Book of Abstracts

of the 77th Annual Meeting of the European Federation of Animal Science

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EAAP

European Federation of Animal Science

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Hamburg, Germany, 07-11 September, 2026

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Welcome to the 77th EAAP Annual Meeting in Hamburg

Ladies and Gentlemen,

on behalf of the Federal Ministry of Agriculture, Food and Regional Identity (BMLEH), the German Society for Animal Production (DGfZ) and the German Organizing Committee are very pleased to invite you to the **77th meeting of the European Federation of Animal Science (EAAP)**, which will take place from **7 to 11 September 2026** in the lively and historic city of **Hamburg**. Under the general theme "**Shaping resilient livestock systems is key to circular agriculture**", we will discuss innovative approaches and solutions for sustainable and resilient livestock systems. Knowledge-based innovations in the livestock sector are essential for developing resilient, circular agricultural systems that can ensure food security, promote animal health, mitigate the negative effects of climate change and contribute to the achievement of the Sustainable Development Goals.

Not only from Europe, but from all over the world, top-class scientists from various disciplines come together at the EAAP Annual Meetings to exchange ideas, present their latest research findings in 12 Study Commissions and establish new contacts from science and industry. No other conference in the world offers such a scientific exchange and excellent platform in this specialized field as the EAAP conferences. We look forward to welcoming around 1,600 – 1,700 participants from over 60 nations to Hamburg.

Hamburg – the "Pearl of the North" – offers an inspiring setting for the conference with its unique blend of historical architecture and modern flair. The city, characterized by its location on the river Elbe, is a cultural jewel. The Speicherstadt, Hamburg City Hall, the Miniatur Wunderland and over 2,500 town bridges invite you to discover them.

Germany is one of the most important agricultural countries in Europe, with animal husbandry playing a central role. Germany's agricultural diversity is matched by its culinary delights. Its beer culture and the enormous range of different styles of bread are internationally known and highly appreciated. Not to forget the popular German wines.

The conference will be complemented by various social events. Look forward to an unforgettable German Evening during Hamburg Harbour Night – set aboard a ship in one of Europe's largest seaports, featuring hearty regional fare and a truly authentic maritime atmosphere.

Discover the beauty of Northern Germany as well. The Technical Tours offer you plenty of opportunities to do so. Or you may be interested in visiting the Hamburg Wadden Sea National Park, which has been a UNESCO biosphere reserve since 1992 and part of the Wadden Sea World Heritage Site since 2011. Immerse yourself in its stunning landscapes and learn why this area is considered one of the most important tidal ecosystems in the world.

We hope you feel inspired and will be able to attend this important conference and join us in shaping the future of animal husbandry and agriculture.

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The European Federation of Animal Science (EAAP)

The main aims of the EAAP are to promote, by means of active co-operation between its members and other relevant international and national organisations, the advancement of scientific research, sustainable development and production systems; experimentation, application and extension; to improve the technical and economic conditions of the livestock sector; to promote the welfare of farm animals and the conservation of the rural environment; to control and optimise the use of natural resources in general and animal genetic resources in particular; to encourage the involvement of young scientists and technicians. More information on the organisation and its activities can be found at www.eaap.org.

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The European Federation of Animal Science (EAAP) has close established links with its sister organizations of American Society of Animal Science (ASAS), American Dairy Science Association (ADSA), Canadian Society of Animal Science (CSAS) and Asociación Latinoamericana de Producción Animal (ALPA) and is also member of the World Association for Animal Production (WAAP).

Industry members

EAAP started in 2023 a new initiative to create closer connections between European livestock industries and the animal science network. Therefore, the “EAAP Industry Club” was shaped with the specific aim of bringing together the important industries of the livestock sector with our European Federation of Animal Science. All companies dealing with animal production (nutrition, genetic, applied technologies, etc.) are invited to join the “EAAP Industry Club” because industries will have opportunity to increase their visibility, to be actively involved in European animal science activities, and to receive news and services necessary to industries. In addition, through the Club, industries will enlarge their scientific network and will receive specific discounts on sponsoring activities.

The Industries that already joined the “EAAP Industry Club” are:

The Club gives:

Visibility • Company name and logo at EAAP website and all relevant documents • Slides with name and logo at Official Events • Priority links with EAAP Socials • Invite, through EAAP dissemination tools and socials, people to events organized by your company • Information disseminated through a brand new Industry Newsletter • Networking • Joining the Study Commissions and Working Groups • Suggest topics to be considered for Annual Meetings Scientific Sessions • Organize Professional Panel through the EAAP platforms • One free registration to each Annual Meeting and at every meeting organized by EAAP • Five individual memberships at no cost • Many possible discounts (-30%) to sponsor and to increase company visibility through: EAAP Newsletter, EAAP website, EAAP Annual Meetings and workshops • Support young scientist by sponsoring scholarships named by the company • Co-Organize and sponsor webinars

Make yourself more visible within the livestock industry via the animal science network!
For more information, please contact eaap@eaap.org.

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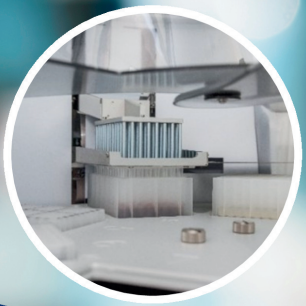

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Young EAAP

What is the YoungEAAP?

YoungEAAP is a group of young scientists organized under the EAAP umbrella. It aims to create a platform where scientists during their early career get the opportunity to meet and share their experiences, expectations and aspirations. This is done through activities at the Annual EAAP Meetings and social media. The large constituency and diversity of the EAAP member countries, commissions and delegates create a very important platform to stay up-to-date, close the gap between our training and the future employer expectations, while fine-tuning our skills and providing young scientists applied and industry-relevant research ideas.

Committee Members at a glance

- Ines Adriaens (President)
- Jana Obsteter (Vice-President)
- Giulia Gislon (Secretary)
- Franziska Koch (National representative)

YoungEAAP promotes Young and Early Career Scientists to:

- Stay up-to-date (i.e. EAAP activities, social media);
- Close the gap between our training and future employer expectations;
- Fine-tune our skills through EAAP meetings, expand the special young scientists' sessions, and/ or start online webinars/training with industry and academic leaders;
- Meet to network and share our graduate school or early employment experiences;
- Develop research ideas, projects and proposals.

Who can be a Member of YoungEAAP?

All individual members of EAAP can join the YoungEAAP if **under 38 years of age**.
Just request your membership form (info@eaap-youngclub.org) and become member of this network!!!

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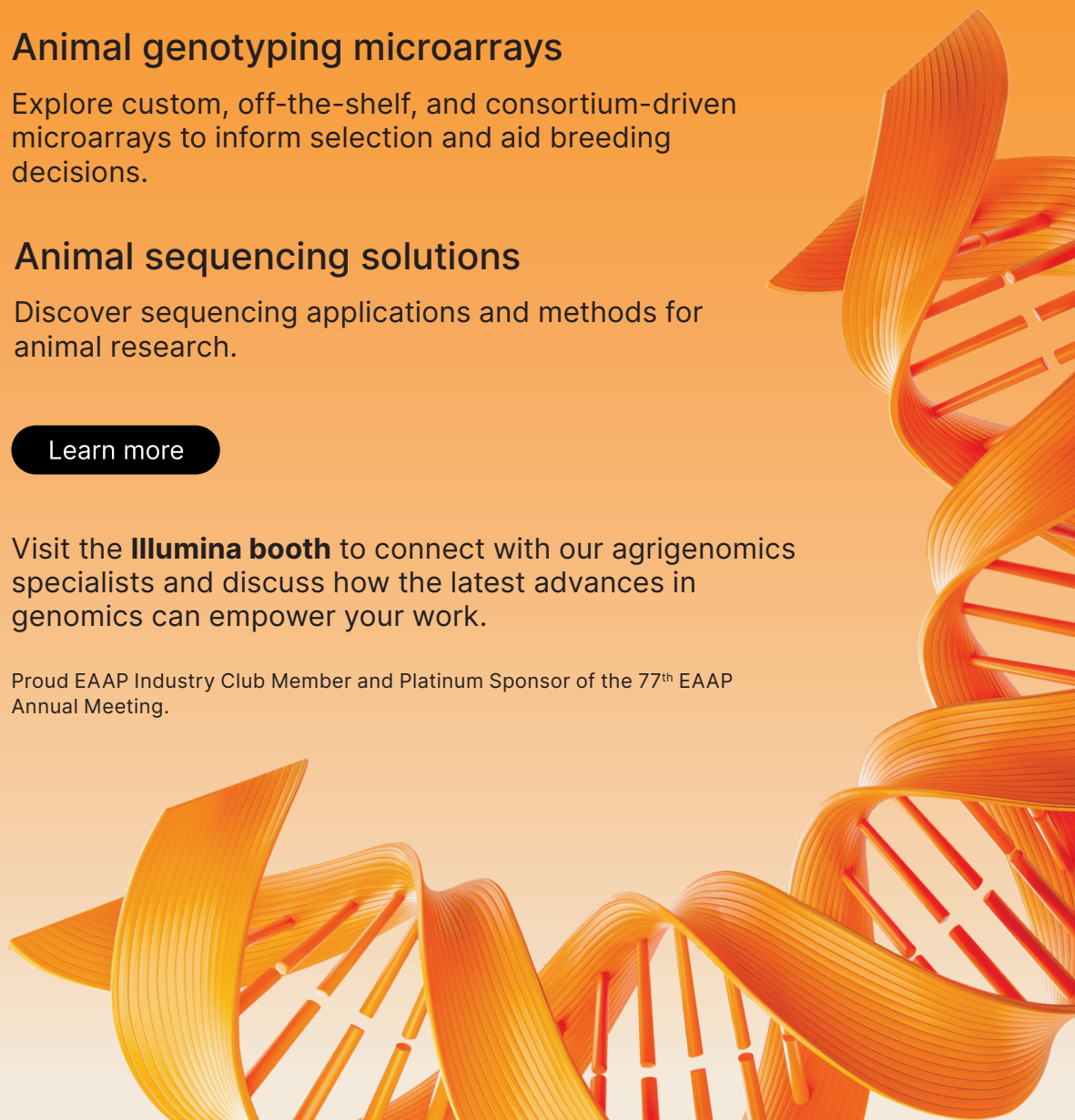

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Scientific programme

EXPLANATORY NOTES ON SESSIONS
Abbreviations after the title are (Organising Commission; Session Type)

Commissions are:
Cattle (C), Genetics (G), Health and Welfare (H+W), Horse (H), Insect (I), Livestock Farming Systems (LFS), Nutrition (N), Pig (P), Physiology (Ph), Poultry (Po), Precision Livestock Farming (PLF), Sheep and Goat (S+G).

Session types are:
Theme Sessions are on key topics in animal science and consist of invited and offered papers.
Industry sessions are led and supported by Industry and comprise invited and offered papers.
Free communications/Bottom-Up: these sessions will be created from submitted abstracts.
Discovery Sessions are invited single/multiple presentations on hot/emerging topics.
Challenge Sessions are free format (workshop, open committee, round table) to debate important issues, not theatre paper presentation.
Early-career scientist's Session.
Project session are sessions based around a project which wants to share its results, often combined with submitted abstracts.
Working Group sessions are the results of an EAAP working group.

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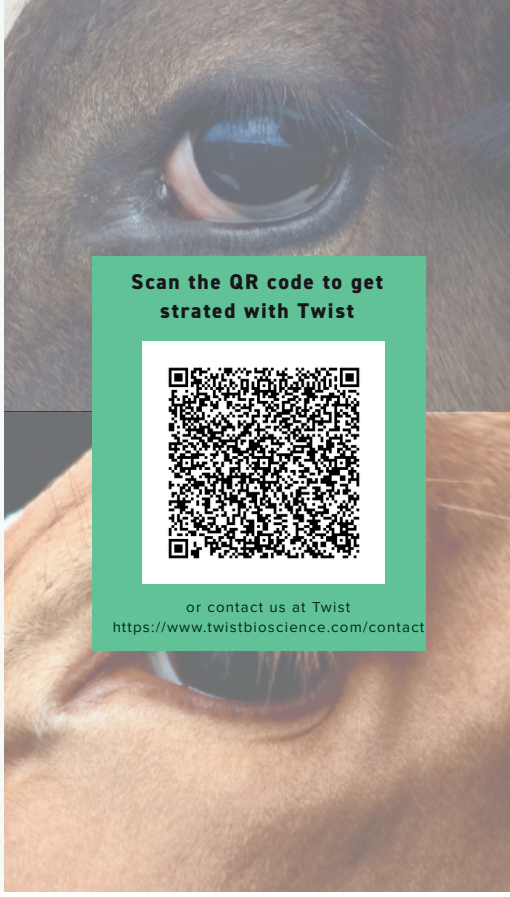

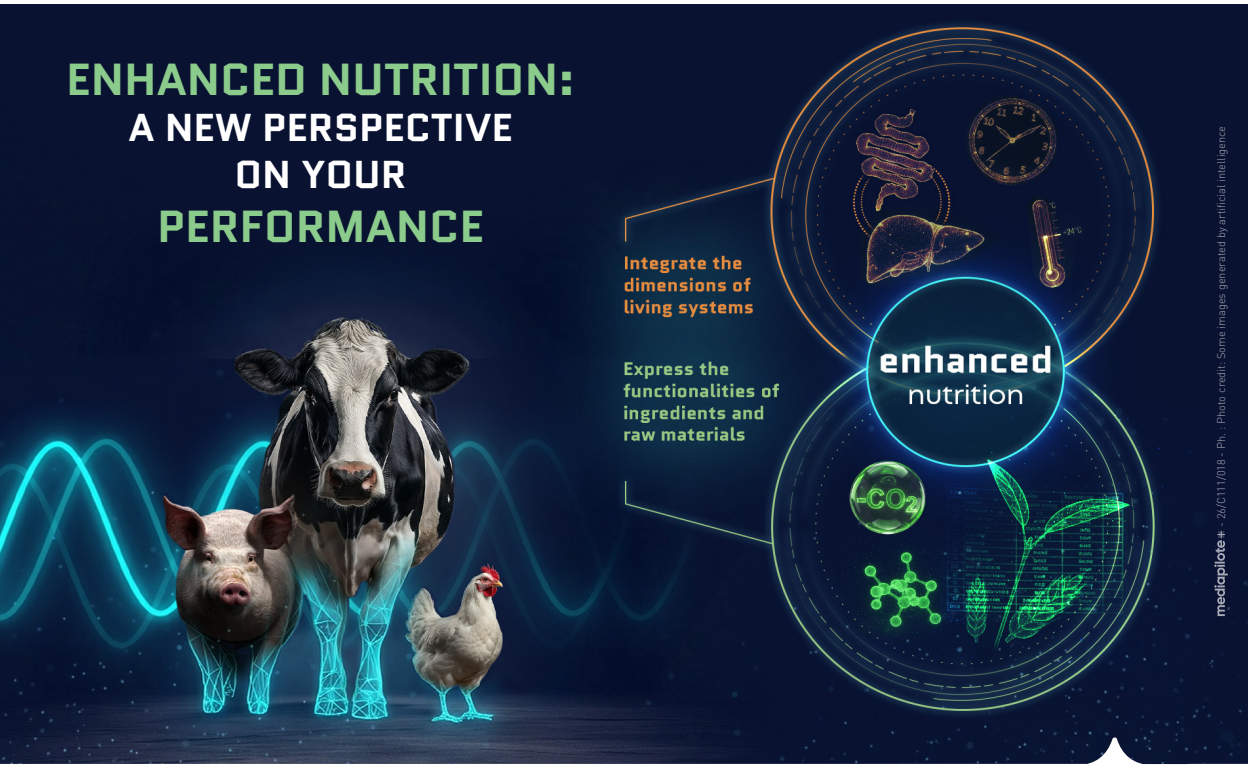

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Theater Presentations

Leanne van de Goor (PhD)
Session 23 | 07/09 | 14:15 - 18:00h | abstr. no 1252314000
'Targeted sequencing approach for equine STR and SNP genotyping to enable transition from STR to SNP-based Equine parentage verification'

Kitty Huijben
Session 72 | 10/09 | 08:30 - 12:30h | abstr. no 1252316182
'Evaluation of targeted sequencing as a flexible alternative to SNP arrays for routine cattle genotyping'

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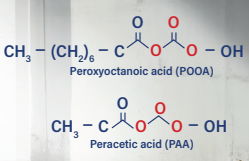
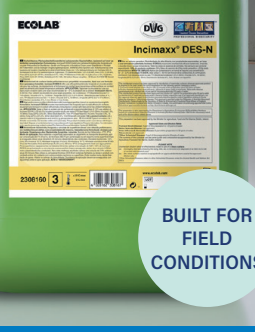


Peroxyoctanoic acid (POOA)

Peracetic acid (PAA)

SYNERGY
Superior oxidative power and efficacy vs. single-acid systems



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Session 1. Breeding for reduced methane, feed efficiency, resiliency and its potential consequences

Date: Monday 7 September 2026; 8:30 - 12:30
Chair(s): Pszczola, Egger-Danner - Commission(s): G - Session Type: Industry session

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Date: Monday 7 September 2026; 8:30 - 12:30
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Chair(s): Ranilla García, Ottoboni, Foggi - Commission(s): N - Session Type: Theme session

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Date: Monday 7 September 2026; 8:30 - 12:30
Chair(s): Manaig, Alvarez-Rodriguez - Commission(s): N - Session Type: Theme session

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Date: Monday 7 September 2026; 8:30 - 12:30
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Date: Monday 7 September 2026; 14:15 - 18:00
Chair(s): Ranilla García, Ottoboni, Foggi - Commission(s): N - Session Type: Theme session

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Chair(s): Slootmans, Díaz de Otálora - Commission(s): PLF, PH - Session Type: Theme session

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Date: Wednesday 9 September 2026; 8:30 - 11:30
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Date: Wednesday 9 September 2026; 8:30 - 11:30
Chair(s): Martin, Berri - Commission(s): C, PO, P, LFS - Session Type: Project session

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Chair(s): Vial, Mathieu

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Date: Wednesday 9 September 2026; 8:30 - 11:30
Chair(s): Emsen, Karatzia - Commission(s): SG, PLF - Session Type: Theme session

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Session 56. Optimal and sustainable insect production

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Date: Wednesday 9 September 2026; 14:15 - 17:45
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Date: Wednesday 9 September 2026; 14:15 - 17:45
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Date: Thursday 10 September 2026; 8:30 - 12:30
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Session 73. EGF session: Resilience of grasslands systems for sustainable livestock production

Date: Thursday 10 September 2026; 8:30 - 12:30
Chair(s): Sturaro, Schneider, Poulopoulou - Commission(s): LFS, SG, C - Session Type: Theme session

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Date: Thursday 10 September 2026; 8:30 - 12:15
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Date: Thursday 10 September 2026; 8:30 - 12:30
Chair(s): Pinotti, Ranilla García, Ottoboni - Commission(s): N - Session Type: Theme session

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Date: Thursday 10 September 2026; 8:30 - 11:30
Chair(s): Vanselow, Kenny - Commission(s): PH - Session Type: Theme session

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Session 77. Carbon accounting on farms: Between standardisation and site-specific realities

Date: Thursday 10 September 2026; 8:30 - 12:00
Chair(s): Rivero - Commission(s): LFS - Session Type: Theme session

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Chair(s): Lawlor, Trevisi, Girard - Commission(s): P - Session Type: Theme session

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Session 79. Advancing Animal Welfare Assessment with PLF Technologies: Insights from the aWISH Project and related research - Part 1

Date: Thursday 10 September 2026; 8:30 - 12:30
Chair(s): Holinger, Maselyne, Blanco-Penedo, Van Noten - Commission(s): HW, SG, PLF, PO, P, G - Session Type: Theme session

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C. Berg

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Session 80. Free Communications on Pigs

Date: Thursday 10 September 2026; 8:30 - 12:00
Chair(s): Trevisi - Commission(s): P - Session Type: Free communications (bottom-up)

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Date: Thursday 10 September 2026; 14:15 - 18:00
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Chair(s): Giagnoni, Valldecabres - Commission(s): C, N - Session Type: Theme session

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Date: Thursday 10 September 2026; 14:15 - 17:30
Chair(s): Pinotti, Ranilla García, Ottoboni - Commission(s): N - Session Type: Theme session

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Chair(s): Kar, Kreuzer-Redmer - Commission(s): N, HW - Session Type: Theme session

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Date: Thursday 10 September 2026; 14:15 - 18:00
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Date: Thursday 10 September 2026; 14:15 - 15:45
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Impact of Methane Data Processing Pipelines on Genetic Variance Component Estimation

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Accurate methane phenotypes are essential for studying the genetic basis of methane emissions in dairy cows. However, methane concentration data collected with sniffers require considerable processing before they can be converted into reliable phenotypes. As a result, different data-processing pipelines are develop to handle the data. This leads to the question if these pipelines lead to meaningful differences in the resulting phenotypes and, consequently, in their genetic parameters estimated The objective of this study was therefore to estimate genetic parameters, including genetic correlations, for methane concentration using three different phenotype-processing pipelines, and to evaluate how the choice of pipeline influences the estimation of genetic variance components. The dataset consists of approximately 8,000 Dutch Holstein cows with about 90,000 weekly methane concentration records collected between 2018 to 2023 across 72 farms in the Netherlands using sniffer-based equipment. Several methane phenotypes were derived using three data-processing pipelines that differed in filtering criteria, alignment steps, and assumptions regarding visit length, background, etc. Each phenotype will be analyzed with a mixed model in ASReml to estimate heritability, genetic variance components, and genetic correlations among phenotypes. The results are expected to show that the choice of data-processing pipeline might affect the magnitude of genetic variance and, consequently, the estimated heritabilities. These findings will highlight the importance of harmonizing methane data-processing procedures in genetic evaluations and demonstrate how different phenotype constructions may affect the genetic parameters for methane emissions. This study is part of the Global Methane Genetics initiative, which is led by Wageningen University and Research and in partnership with the Bezos Earth Fund and the Global Methane Hub.

Genetic parameters for longitudinal CH_4 and CO_2 concentrations from sniffer sensors in automatic milking systems

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Automated milking systems (AMS) combined with emission sensors enable continuous, high-frequency recording of cow performances and enteric CH_4 and CO_2 emissions under commercial farm conditions. The objective of this study was to use emission phenotypes from continuous AMS-sniffer measurements to estimate genetic parameters for CH_4 and CO_2 concentrations per AMS visit as well as for daily and weekly averages. Data were recorded in three commercial AMS farms in Germany between August and December 2025. Cow emissions were obtained from Moologer gas analysers (sniffer), installed in 12 Lely-Astronaut AMS. After merging the longitudinal AMS production and sniffer data, 122,007 records representing the informative AMS visits from 774 Holstein cows were available for the ongoing analyses. CH_4 and CO_2 concentrations (in ppm) per visit were further averaged per day and week yielding 58,111 and 14,923 records, respectively. Genetic parameters were estimated by applying repeatability (RM) and random regression models (RRM). For the RM, fixed effects included the parity and a regression on days in milk (DIM). Random effects included the combined herd-robot-test day effect to account for systematic differences between AMS/sniffer units and recording days, the additive genetic and the permanent environmental effects. The CH_4 repeatabilities were 0.32 ± 0.014, 0.47 ± 0.015 and 0.58 ± 0.015 for averages per visit, per day, and per week, respectively. Moderate repeatibilities were also estimated for CO_2 with 0.40 ± 0.015 (per visit), 0.50 ± 0.015 (per day) and 0.59 ± 0.015 (per week), indicating consistency of cumulative emission measurements with progressing time. Heritabilities for CH_4 from the RM were 0.24 ± 0.040 (per visit), 0.31± 0.049 (per day) and 0.30 ± 0.065 (per week), and heritabilites for CO2 0.22 ± 0.050 (per visit), 0.26 ± 0.052 (per day) and 0.27 ± 0.065 (per week). Aggregating emissions to daily and weekly measurements implied increased repeatabilities and heritabilities, due to the elimination of short-term "environmental noise" reflected through declining residual variances. The RMM considered the fixed effects from the RM and in addition, random regression coefficients for the additive-genetic and permanent environmental effects along DIM, modelled via Legendre polynomials of order 4. The quartic model presented moderate additive-genetic variation for CH_4 with h^2 ranging from 0.12 to 0.15 in the course of lactation. Genetic correlations between CH_4 from different time points were close to 1 for neighbouring DIM, and were lowest between early and mid-lactation with a minimal coefficient of -0.13 between CH_4 at days 1 to 5 and day 150. The genetic correlations from the multi-trait RM (simultaneously considering sniffer emission and AMS production traits) were 0.73, -0.04 and 0.05 between CH_4 with daily milk yield, protein content and fat content, respectively. Hence, selection for increased milk yield implies a proportional increase of methane concentrations. The genetic correlation between CH_4 and CO_2 was 0.98, indicating a shared genetic background for both emission traits. The dense sniffer data structure from AMS enabled RRM applications to study genetic effects for CH_4 and CO_2 concentrations along the time trajectory. The moderate and accurate heritability estimates indicate the potential of selection on reduced emissions, but it is imperative in ongoing studies to infer genetic covariances with further breeding goal traits during lactation.

Genetic parameter estimation for methane (CH4), carbon dioxide (CO2) and oxygen (O2) production in first parity Holstein cows

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Methane (CH4) and carbon dioxide (CO2) emitted by cows during eructation are key greenhouse gases (GHG) contributing to global warming. Methane has a short atmospheric lifetime, but its impact on climate change is acute, and actions to reduce its emissions will have a near-term effect. In contrast, CO2 has a longer atmospheric lifetime, and efforts to reduce its emissions are a long-term goal to decrease global temperatures. Systems like GreenFeed and sniffers that measure CH4 emissions also record CO2 production simultaneously. Despite the availability of CO2 records, research on genetic parameter estimates and their possible correlations with CH4 and other important economic traits remains limited. Thus, the objective of this study was to estimate genetic parameters for CH4 (g/day), CO2 (g/day), and O2 (g/day) production and to characterize the relationship among these traits. Records of CH4, CO2, and O2 production were measured in first-parity Holstein cows between 110 and 180 days in milk using a Greenfeed machine. Cows were sampled for 5 consecutive days (Monday to Friday), four times a day at 0800 h, 1200 h, 1600 h, and 2000 h (2017 to 2020) and 3 times per day at 0800 h, 1200 h, and 1600 h (2020 to 2025). The recording time length ranged from 2 to 20 minutes. After data cleaning, only cows with two or more records per day and at least one day of records were retained. Outliers were removed if values were 3 standard deviations above or below the mean. For each animal, records were averaged across the 5-day recording period to derive the final phenotype. Univariate and bivariate animal models were fit in ASREML. Only significant ($p < 0.05$) environmental fixed effects were included in the models. For CH4, the model included season of record (Srecord; four levels: 1 – from Jan to Mar; 2 – from April to June; 3 – from July to Sept; and 4 – from Oct to Dec) and year-season of calving (YScalving). For CO2, the effects of year-season of record (YSrecord) and YScalving were included in the model; for O2, the effects of year of record and YScalving were included. Heritabilities estimated were 0.44 (0.08), 0.36 (0.09), and 0.41 (0.08) for CH4, CO2, and O2 production, respectively. Genetic correlations were 0.70 (0.09) between CH4 and CO2 and 0.64 (0.10) between CH4 and O2. The CO2-O2 bivariate model did not converge. However, the correlation between the estimated breeding values from the univariate analyses was 0.90, suggesting a high underlying genetic correlation that may have hindered convergence. Phenotypic correlations were 0.68 (0.02) for CH_4–CO_2 and 0.61 (0.03) for CH_4–O_2. Residual correlations were 0.67 (0.05) for CH_4–CO_2 and 0.58 (0.06) for CH_4–O_2. The moderate heritabilities and strong genetic correlation between CH4 and CO2 indicate that selection to reduce one GHG gas is also likely to reduce the other. Because CH4 and CO2 can be measured concurrently with GreenFeed systems or sniffers on farms, these traits are promising candidates for inclusion in a multi-trait index designed to select animals that produce less GHG.

Genetic parameters of sniffer-based enteric methane concentration in dairy cows

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Enteric methane (CH4) emissions from dairy cattle contribute substantially to greenhouse gas emissions, and genetic mitigation requires reliable phenotypes and robust parameter estimates for accurate breeding values. The objective of this study was to estimate variance components for sniffer-based CH_4 emissions in dairy cows and to evaluate the robustness of these estimates across different approaches of data-quality filtering. Records were collected from automatic milking systems (AMS) equipped with sniffers in Holstein Danish dairy herds, including up to 9,979 cows from 22 commercial farms. The raw data was processed using the Gas Emission Data Alignment (GEDA) pipeline, then the four study datasets were generated using variable data-quality filtering thresholds, where the threshold values are the local repeatability estimates calculated within processed sub-sets of data (no filtering, ≥30%, ≥35%, and ≥40%). As a result, the produced datasets differs by numbers of animals, farms, and recording units. Three methane phenotype definitions were evaluated: (1) the mean methane concentration per visit (ppm) (2) the daily mean methane concentration per cow, calculated by averaging all sensor observations recorded within a day (ppm), (3) Weekly methane phenotypes (ppm) were subsequently derived by correcting daily records for herd, AMS, sniffer, and day effects, then averaging the corrected values within weeks in milk (WIM). Repeatability models were fitted to daily, weekly, and per-visit methane phenotypes to enable direct comparison of genetic parameters across phenotype definitions. Linear mixed models included parity, herd × year × month × sniffer × AMS, and lactation stage effects (DIM and DIM² for daily and visits records, or WIM and WIM² for weekly records, together with the Wilmink function) as fixed effects, with additive genetic and permanent environmental effects modelled as random effects. Across all datasets, CH4 concentration exhibited low to moderate heritability, ranging from approximately 0.11–0.15 for per-visit phenotypes, 0.13–0.16 for daily phenotypes, and 0.22–0.23 for weekly phenotypes. As expected, repeatability increased, reaching the highest values for weekly methane phenotypes (0.57–0.62), compared with per-visit (0.27–0.39) and daily (0.32–0.41) phenotypes. Stricter data filtering reduced residual variance and increased thereby heritability and repeatability, while additive genetic variance estimates remained relatively stable across datasets. These results demonstrate that sniffer-based methane concentration traits are heritable, with heritability estimates increasing when stricter GEDA-based data-quality filtering criteria were applied. Repeatability was consistently higher than heritability, indicating a substantial contribution of permanent cow-specific effects. Overall, these findings support the feasibility of including sniffer-based methane phenotypes in breeding programs aimed at reducing the environmental footprint of dairy cattle.

Exploring the uniqueness of the genetic architecture of estimated methane emissions in Angler cattle based on existing prediction equations

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Methane emissions (CH4) vary among individuals and breeds and showed moderate heritability across studies making breeding strategies promising to achieve sustainable, cumulative and permanent reduction in CH4 in dairy cattle. Genome-wide association studies (GWAS) are gold standard to identify genomic regions associated with specific phenotypes such as CH4. Mid-infrared (MIR) spectra obtained from routinely collected milk samples in dairy cattle on a large scale can be used for the quantification of individual CH4. However, most research has been conducted in the Holstein Friesian (HF) breed, whereas there is a lack of studies for small local German breeds like Angler (RVA) concerning climate efficiency and the potential of breeding strategies to reduce CH4. Thus, this study aims to investigate the genetic background of estimated CH4 in RVA. Data from 2,451 cows (1,901 RVA; 550 HF) across lactations one to seven from sixteen farms in Northern Germany were available. Eight farms kept both breeds, enabling a comparison of the results between breeds. Given the limited number of HF animals in the sample, findings were additionally cross-checked against existing literature. Monthly milk samples from June 2023 to June 2025 were analysed and individual CH4 were calculated using two prediction equations based on MIR spectra respectively on fatty acids predicted from MIR spectra, resulting in slightly different mean values. The average CH4 (± SD) was 459.27 ± 69.59 g/d for RVA (467.94 ± 71.83 g/d for HF) calculated according to a modified version of the equation distributed by European Milk Recording and 404.69 ± 113.47 g/d for RVA (416.22 ± 125.14 g/d for HF) calculated according to the equation of the FBN Dummerstorf. Thus, the estimated average CH4 was slightly lower for RVA, regardless of the prediction equation applied. After filtering the genotype data for MAF < 0.01 and SNP call rate < 0.99 40,405 autosomal SNPs remained for RVA and 43,590 for HF, respectively. To identify the underlying genetic background, variance components were estimated using genomic restricted maximum likelihood (GREML) and GWAS were conducted using the tool GCTA with predicted CH4 for distinct lactation months (1-12) as phenotypes. Mixed linear models (MLMA) with fixed effects for lactation number and herd-year-month were used after prior testing of possible factors. In accordance with previous studies, heritability estimates ranged from 0.10 (± 0.04 SE) to 0.37 (± 0.06 SE) for RVA depending on the CH4 trait and lactation month and were mostly significant ($p < 0.001$). First GWAS results indicate promising genomic regions with significant SNPs as well as differences in the association patterns between traits and within traits across lactation. Further analyses are conducted to explore the genetic architecture and differences between breeds as well as potential interactions with different traits. Since the prediction equations do not yet specifically consider local breeds like RVA, methane measurement data is currently collected for RVA to validate or modify the equations for CH4 to improve subsequent analyses.

Phenotypic and genomic analyses of real and MIR-predicted methane phenotypes in Australian dairy cattle

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Methane emissions from dairy cattle are a major contributor to agricultural greenhouse gas (GHG) emissions. The current technologies (i.e., respiration chamber) are expensive and not scalable for phenotyping of methane. Milk mid-infrared (MIR) spectroscopy data is a high-throughput and cost-effective phenotyping tool and has emerged as a promising proxy phenotype for methane production. This study evaluated the potential of milk MIR spectra as a proxy for methane (Me) measurement in Australian dairy cattle and subsequently applied it in genomic prediction. We first benchmarked machine learning (ML) algorithms, including random forests, support vector machines, and artificial neural networks, with traditional partial least squares regression (PLSR) in phenotypic prediction of methane using milk MIR. The PLSR model then was applied to evaluate the phenotypic prediction of methane using directly measured methane and milk MIR phenotypes from 224 cows from Agriculture Victoria's Ellinbank SmartFarm (ELB) and 12,057 commercial (COMM) cows. We followed the routine protocol to pre-process MIR data and calibrated the prediction models to improve phenotypic prediction accuracy. The prediction model fitting the combination of 521 MIR spectra and parity or parity and milk yield was the best-performing models for methane production, achieving predictive accuracy up to r = 0.60. Heritabilities for MIR-predicted methane traits were estimated high with large standard errors due to a small size of training population (eg. $h^2 \approx 0.71$, s.e.= 0.17 using predicted phenotypes from model MIR and parity for ELB cows). Genetic correlations between real and predicted phenotypes reached 0.75 (s.e.=0.238) within ELB and 0.24 (s.e.=0.137) across COMM cows using bivariate regression analyses. Genomic prediction results using GBLUP showed that using MIR-predicted methane achieved a similar prediction accuracy to using directly measured methane. While the robustness of the Australian methane breeding value is yet to be improved, which requires a larger training population, MIR demonstrates promise for large-scale methane prediction and genomic evaluation for Australian dairy systems, offering a practical alternative to expensive direct measurement methods.

Rumen metabolites as potential indicators to mitigate methane emissions in dairy cattle

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Although multiple mitigation strategies have been proposed to reduce enteric methane (CH4) emissions from ruminant animals, methane production remains a major environmental challenge for the cattle sector. Genetic selection for reduced enteric CH_4 emission represents a promising and permanent mitigation strategy. However, accurate estimation of breeding values requires large-scale phenotyping, and direct CH_4 measurements are costly and typically limited to farms equipped with specialized infrastructure, such as automatic milking systems fitted with sniffer devices. Hence, cost-effective and biologically informative indicator traits are needed. Proxies related to rumen function, such as rumen microbial composition, fermentation pathways, and associated metabolites, offer mechanistic insight into methanogenesis at the molecular level and may capture host genetic variation underlying CH4 production. In particular, rumen metabolites involved in fermentation pathways could serve as practical indicator traits for mitigating CH4 emissions through selective breeding in dairy cattle. However, the extent to which host genetics regulates these pathways and their associated metabolites remains insufficiently characterized. This study evaluates the potential of rumen metabolites as indicators for enteric CH_4 emissions by estimating their heritabilities and their correlations with CH4 emissions. CH4 emissions were measured in approximately 500 Holstein dairy cows using the sniffer method, and CH4 concentration was used as the CH4 phenotype. Blood and rumen samples were collected and sent to the laboratory for NMR spectroscopy. The NMR spectra files from the lab were pre-processed in R using the AlpsNMR package, and metabolites in the rumen and serum samples were annotated using the ASICS package. Annotated metabolites were log-transformed before genetic analysis. Genetic variances and heritabilities of metabolites and CH4 phenotype were estimated using a single-trait animal model with parity as a fixed effect and Days in Milk as a covariate. Metabolites with heritability estimates greater than 0.05 were used to estimate genetic correlation with CH4 phenotype. Genetic and phenotypic correlations were estimated using a bivariate animal model with the same fixed effects and covariates as in the single trait model. 109 and 76 metabolites were annotated for serum and rumen, respectively. For serum, heritability estimates were very low (≤0.04) for 27 metabolites, low (0.05 to 0.19) for 61 metabolites, and moderate (0.20 to 0.38) for 21 metabolites. For rumen metabolites, heritability estimates were very low (≤0.04) for 43 metabolites, low (0.05 to 0.19) for 27 metabolites, and moderate (0.22 to 0.34) for 6 metabolites. In serum metabolites, Methylmalonic acid (MMA) recorded the highest heritability, while Malonate recorded the highest and lowest heritability in rumen metabolites. Of the 33 rumen metabolites used to estimate genetic correlation with the CH4 phenotype, 11 had negative genetic correlations (-0.36 ± 0.40 to -0.06 ± 0.51), and 22 had positive genetic correlations (0.03 ± 0.26 to 0.67 ± 0.54). The results of this study revealed that some rumen metabolites are heritable and are potential indicators for enteric CH_4 emissions. Furthermore, metabolites that were positively and negatively correlated with CH4 are known to be associated with hydrogen consumption and production pathways in the rumen. This study demonstrates the potential of rumen metabolites as indicator traits for enteric CH4 emissions in dairy cows, highlighting their suitability for large-scale phenotyping and their application in genetic selection programs aimed at mitigating methane emissions.

Genetic determinism of feed and water intakes and resilience traits in divergently selected RFI Romane sheep

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Improving feed efficiency is a key objective in animal production to reduce feed consumption and waste without compromising performance. Residual Feed Intake (RFI) is the most relevant criterion, as it identifies animals that eat less than expected for their production and maintenance needs. However, accurate RFI estimation requires individual feed intake measurements, which, in ruminants, depend on technically complex equipment, limiting large-scale phenotyping and selection. In contrast, individual water intake can be recorded using simpler devices, enabling the phenotyping of about four times more animals than forage intake systems. At INRAE P3R (https://doi.org/10.15454/1.5483259352597417E12), concentrate (ad libitum or restricted), forage, and water intakes for groups of 25 small ruminants can be recorded simultaneously during a single experiment. Since 2015, a divergent selection experiment on RFI under a 100% concentrate diet has been conducted in Romane meat sheep, based on young males' phenotypes. Between 2022 and 2025, around 640 sheep (350 males and 290 females) from the fourth to sixth generations were phenotyped. Males were recorded in spring for six weeks between 3 and 5 months of age under a 100% concentrate diet (Phase I) to establish the lines. The same males and their sisters were then recorded for six additional weeks between 6 and 8 months of age under a mixed diet with forage offered ad libitum and restricted concentrate (720 g for males, 660 g for females) in Phase II. Water intake was recorded during both Phase I and II. From daily intake (DI) records— of concentrate, forage, and water consumption—two resilience indicators were derived. First, the classical log-transformed coefficient of variation of daily intake, logCV(DI), was calculated. Second, the log-transformed variance of the area under the curve, logVar(AUC), was computed as a "consistency" criterion, as proposed by Cavani et al. (2023). Unlike logCV(DI), logVar(AUC) is independent of the total amount ingested and reflects the stability of daily intake fractionation patterns over time. The differences between the divergent RFI lines and the variance components were estimated for three traits: average daily intake (ADI), logCV(DI) and logVar(AUC), for the 4 ad libitum delivered feed (concentrate and water intakes during Phase I, and for forage and water intakes during Phase II). During early growth (Phase I), males consumed on average 2.28 ± 0.30 kg of concentrate and 6.68 ± 1.40 L of water per day. In Phase II (summer), water intake averaged 6.50 ± 1.45 L/day in males and 4.96 ± 0.96 L/day in females, while forage intake reached 1.24 ± 0.22 kg/day in males and 1.09 ± 0.19 kg/day in females. The line effect was highly significant for all intakes: in Phase I, RFI− males consumed 193 g less concentrate and 0.998 L less water per day than RFI+ males. In Phase II, efficient animals of both sexes consumed 110 g less forage and 0.764 L less water per day. Line effect was generally not significant for logCV(DI), except when calculated from water intake in Phase II, whereas a systematic difference was observed for logvarAUC, with higher values in efficient animals across all feed types. In males, ADI heritability ranged from 0.42 ± 0.10 (concentrate) to 0.68 ± 0.11 (water) in Phase I, and reached 0.59 ± 0.10 (forage) and 0.78 ± 0.11 (water) in Phase II. Resilience indicators showed null heritability for concentrate intake in Phase I, but reached 0.26 ± 0.10 and 0.22 ± 0.12 (consistency criterion) and 0.41 ± 0.11 and 0.44 ± 0.12 (logCV(DI)) in Phase II for forage and water intake, respectively.

Circadian feeding patterns in Swiss pigs are highly heritable and associated with feed efficiency

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Circadian eating patterns are the recurrence of eating behaviour along a 24-hour cycle and are thought to be controlled by the hypothalamus, stimulated by environmental factors such as light cycles. Pigs' feeding behaviour can be an important indicator of their performance, health and welfare but monitoring feeding patterns is challenging in livestock. However, the introduction of automated feeding stations now enables to study circadian eating patterns in depth. These data indicate that feed intake in pigs follows pronounced daily patterns, typically characterized by high intake in the morning and afternoon and minimal intake at nights. Here, we estimate the heritability of circadian feeding patterns and its relationship with feed efficiency in pigs. Feeding station data were obtained from the performance testing station of SUISAG, a Swiss breeding company, for pigs from the Swiss Landrace (SLR; n=2,575), the Swiss Large White dam line (SLWD; n=9,365) and Swiss Large White sire line (SLWS; n=2,687), covering the feeding period between 75 to 155 days of age. Pedigree data were available for all pigs and a subset of the phenotyped cohort had microarray-derived genotypes (SLR: n=1,625; SLWD: n=2,485; SLWS: n=360), which were imputed to the sequence-level. Feeding station records were aggregated to hourly feed intake, giving 24 observations per day and pig. Circadian patterns were quantified using wavelet analysis after de-trending and amplitude correction. For each pig and day, the strength of a circadian rhythm was assessed by comparing observed wavelet results with 200 white-noise simulations. Days with a mean p-value < 0.05 were classified as circadian, and the proportion of days with a circadian rhythm over the 80-day period reflects the strength of pigs' circadian eating patterns. Genetic parameters were estimated separately for each breed using single-step GBLUP implemented in gibbsf90+ software accounting for pen (random), sex and farm of origin (fixed) and the arrival weight and number of daily records (covariate). Genome-wide association studies (GWAS) were performed using GCTA-software, accounting for the same effects as described above and the first four principal components calculated from the genomic relationship matrix. The proportion of days with a circadian pattern showed substantial phenotypic variability, with a mean proportion of 0.46 (SD=0.30). Sex effects were highly significant ($p<0.0001$), with sows exhibiting the highest proportion of circadian days across all breeds followed by boars and barrows. The proportion of circadian days was negatively correlated with average daily feed intake ($r=-0.17$) and feed conversion ratio ($r=-0.21$), but not with daily gain ($r=0.01$), meaning that a stronger circadian feed intake improves feed efficiency. Heritability estimates for the proportion of circadian days were high across breeds (52.0-58.1%). A GWAS identified a QTL for the proportion of days with a circadian pattern on SSC4 for both SLR ($p=1.6\times10^{-8}$) and SLWD ($p=1.2\times10^{-12}$), near two functional candidate genes. Fine mapping is currently ongoing. Circadian eating behaviour showed substantial heritable variation and was favourably associated with feed efficiency, primarily through reduced feed intake without compromising growth. The identification of a shared, highly significant QTL on chromosome 4 in two independent breeds further supports a genetic basis for circadian feeding behaviour and highlights promising candidate regions for future functional analyses.

Plasma metabolomic signatures differentiate Duroc pigs with divergent feed intake: insights into the molecular architecture of a complex trait

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Feed intake is a biologically complex and economically relevant trait in pig production, influencing growth rate, efficiency, sustainability, and overall production costs. Its variability results from the interaction between environmental factors and genetically regulated biological processes operating across multiple molecular and physiological levels. Dissecting the molecular basis of feed intake is therefore essential to better understand its biology and to identify biomarkers that may improve breeding, nutritional strategies and the sustainability of pig production systems. Metabolomics, defined as the comprehensive analysis of low molecular weight molecules in biological systems, enables the exploration of intermediate molecular phenotypes linking genotypes to complex traits, potentially providing simpler and biologically informative proxies. The objective of this study was to identify plasma metabolites discriminating pigs with extreme and divergent feed intake and to evaluate their biological relevance. A total of 290 growing male Duroc pigs were selected from a larger performance-tested population and classified into high (n=150) and low (n=140) feed intake groups. Untargeted plasma metabolomics quantified 756 metabolites. After quality control, 587 metabolites spanning major biological classes were retained. Missing values were handled, generating 25 minimally different datasets. A robust feature selection pipeline based on the Boruta algorithm (Random Forest classifier) was applied across the 25 datasets, combined with 10-fold cross-validation, resulting in 1,250 feature selection runs. Metabolites consistently selected across runs were considered discriminant. Their discriminatory power was further evaluated using the ROC-AUC, principal component analysis (PCA), and standard Random Forest. Pearson correlations with raw feed intake were calculated, and biological interpretation was supported by pathway enrichment and metabolite network analyses. Twenty-nine metabolites (~5% of the filtered dataset) consistently discriminated between the two groups. Most were amino acids (66%), followed by nucleotides (17%), lipids (10%), one carbohydrate, and one unnamed compound. Amino acids were significantly over-represented (PFDR<0.05), whereas lipids were under-represented relative to the whole metabolome. Correlations with feed intake ranged from -0.40 to +0.34. AUC values ranged from 0.61 to 0.75, indicating moderate but consistent discriminatory capacity. PCA based on the 29 selected metabolites improved group separation compared with the whole-metabolome PCA. Pathway analyses highlighted enrichment of glutamate, histidine, branched-chain amino acids, and lysine metabolisms. These results support the hypothesis that specific plasma metabolites reflect biological mechanisms underlying feed intake variability. The predominance of amino acid signatures suggests an active involvement of protein and energy metabolism in differentiating intake extremes. In conclusion, the pig plasma metabolome contains reproducible molecular phenotypes associated with feed intake. These findings contribute to a better understanding of the biological architecture of feed efficiency and may support the development of metabolomics-informed breeding strategies. This study received funding from the European Union's Horizon Europe research and innovation program under grant agreement No. 101059609 (Re-Livestock project).

Functional impact of RNA-Seq derived SNPs associated with feed efficiency in Nellore cattle

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Feed efficiency (FE) is a polygenic trait of major economic importance that promotes resource optimization and economic viability in tropical beef cattle. Although genome- wide association studies have identified numerous quantitative trait loci associated with FE in Nellore cattle, the functional mechanisms driven by specific variants within these regions remain largely unclear. Moving beyond genomic location to transcriptomic function is crucial for refining genomic selection models. This study aimed to identify, functionally annotate, and analyze the metabolic pathways associated with single- nucleotide polymorphisms (SNPs) from RNA-Sequencing (RNA-Seq) data in Nellore cattle with contrasting residual feed intake (RFI) values. The RNA-Seq data from five tissues (liver, muscle, hypothalamus, pituitary gland, and adrenal gland) were analyzed for SNP detection in two groups, being 9 individuals with high RFI and 9 individuals with low RFI. Track tools were used to identify fixed and unique SNPs within each group. Amino acid changes (AAC) and splice effects (SE) were analyzed using CLC Genomics Workbench (Aarhus, Denmark). Functional impact of the identified variants was assessed using the Ensembl Variant Effect Predictor (VEP) based on the ARS- UCD1.3 assembly. Metabolic pathway enrichment analysis was performed using the Kyoto Encyclopedia of Genes and Genomes (KEGG) database via the DAVID software. A total of 4,805 SNPs were identified, of which 2,525 were shared between groups, representing the common genetic background. In the low-RFI group, 1,029 SNPs were identified in overlap with 948 genes, comprising 144 AACs, 11 SEs, 3 variants with both AAC and SE, and 2 high-impact and 344 moderate-impact transcripts. Conversely, the high-RFI group exhibited a higher mutational load, with 1,251 SNPs in overlap with 1,016 genes, including 161 AACs, 14 SEs, and 6 AAC+SE. The high-RFI group presented more high-impact (8) and less moderate-impact (338) transcripts than the low-RFI group. A total of 61 pathways in the low-RFI group and 58 in the high-RFI group remained significant after false discovery rate correction (q < 0.05). Metabolic pathways, oxidative phosphorylation, and carbon metabolism were among the pathways across groups. The low-RFI group showed stronger enrichment in thermogenesis and fatty acid degradation, suggesting optimized energy expenditure. In contrast, the high- RFI group included pathways such as arginine and proline metabolism, indicating distinct nutrient partitioning potentially focused on metabolic maintenance. Interestingly, oxidative phosphorylation involved higher number of associated genes in the low-RFI group (44 vs. 35). This suggests a more robust mitochondrial capacity and fine-tuned energy metabolism in efficient animals, minimizing heat loss while maximizing nutrient utilization. Furthermore, the higher prevalence of high-impact functional variants in inefficient animals suggests a greater genetic burden affecting metabolic stability. These findings provide a functional map of genomic variations, highlighting specific targets to improve genomic prediction for FE in Bos indicus and advancing precision breeding programs for efficient animals.

Genetic correlation between deep phenotypes and measured phenotypes of feed efficiency

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Deep phenotypes are the inner meta-mechanisms of feed efficiency. Using deep phenotypes, such as net energy efficiencies, in breeding may improve feed efficiency more rapidly than the current feed efficiency measures; gross feed efficiency, residual feed intake and feed saved. However, deep phenotypes are difficult and expensive to measure in practice. In earlier studies, deep phenotypes were therefore estimated by using LiGAPS-Dairy (a mechanistic model simulating feed efficiency), a genetic algorithm (optimization algorithm based on natural selection) and measured phenotypes. The measured phenotypes of feed efficiency were weekly fat- and protein-corrected milk yield (FPCM; kg/day), weekly dry matter intake (DMI; kg/day), and average weekly bodyweight (BW; kg), whose genetic correlations to the estimated deep phenotypes were not assessed in those studies. Hence, the present study aims to estimate the genetic correlation (rg) of deep phenotypes with FPCM, DMI, and BW. Eight deep phenotypes were estimated for 1,665 cows from 195,492 weekly FPCM, 56,511 weekly DMI, and 71,856 weekly BW records. The eight deep phenotypes were net energy for maintenance (NEm), intake capacity (Int_cap), maximum adult total body weight (TBW), the three parameters of Wood's lactation curve (W1, W2, W3), net energy efficiency for milk synthesis (NEEFF), and protein efficiency for milk synthesis (PROTEFF). A multivariate linear mixed model was fitted. Estimated rg between deep phenotypes and measured FPCM, DMI, and BW ranged from strongly positive (0.96 ± 0.01) to strongly negative (-0.75 ± 0.10). Similarly, strong positive rg between W2 and FPCM (0.94 ± 0.03), Int_cap and FPCM (0.77 ± 0.06), TBW and DMI (0.79 ± 0.02), TBW and BW (0.96 ± 0.01), and W2 and DMI (0.88 ± 0.03) were found. Negative rg were found between W3 and FPCM (-0.66 ± 0.10), W3 and DMI (-0.75 ± 0.10), PROTEFF and DMI (-0.67 ± 0.04), PROTEFF and BW (-0.31 ± 0.04), and NEEFF and DMI (-0.37 ± 0.06). Overall, selection on deep phenotypes for feed efficiency is expected to produce correlated responses in production traits. Next step is to estimate rg between deep phenotypes and gross feed efficiency, residual feed intake and feed saved. Key words: Deep phenotypes, mechanistic model, genetic correlation, feed efficiency

Genetic analyses of the relationships among milk yield, feed intake, body weight and energy balance in Holstein Friesians using selected SNP subsets

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In modern dairy cattle breeding, the focus has expanded beyond productivity and increasingly encompasses animal health and robustness. In early lactation, dairy cows frequently experience a negative energy balance, where energy needs for milk production and maintenance exceed the energy intake, compromising immune competence, and increasing the incidence of diseases. Previous research has confirmed genetic links among milk yield (MY), dry matter intake (DMI), and energy balance (EB) in dairy cows. Building on these findings, the present study explores these relationships in greater depth, with the goal of revealing underlying biological mechanisms and molecular genetic insights. Using selected SNP subsets, genomic heritabilities and correlations were estimated across lactation for MY, DMI, EB and body weight (BW), to determine the contribution of these subsets to the genetic architecture of the traits. Weekly records for MY, DMI, EB and BW from 3,485 lactations of 2,188 Holstein Friesian cows were available, along with imputed whole-genome sequence data (WGS) containing ~ 17 million SNPs for all animals. WGS data was partitioned into subsets and three subsets were selected for this study: Selection Signatures (SelS, 1,787 SNPs), metabolic QTL (mQTL, 5,102 SNPs) and SNPs used for routine genetic evaluation (50k, 44,315 SNPs). Traits were analyzed with single-trait random regression models, while genomic correlations were estimated with pairwise bivariate random regression models. Heritabilities ranged from low to moderate, with 0.027 (±0.010) for EB to 0.194 (±0.019) for MKG. While heritabilities estimated with the subset 50k were highest, subsets mQTL and SelS explained a considerable proportion of heritability despite their much smaller SNP counts. This indicates a potentially functional and biological significance of these genomic regions for the traits under study. The subset SelS contains functional SNPs that occur significantly more frequently in dairy cattle, thereby capturing relevant genetic variation for the investigated traits. The subset mQTL includes SNPs associated with milk fatty acid concentrations and is predominantly located in functional genomic regions marked by methylation. Since these variants are closely linked to metabolic processes and milk fat synthesis, particularly during phases of negative energy balance, they capture genetic effects directly related to MY, DMI, and EB. Genomic correlations ranged from −0.285 (±0.109) between MY and EB to 0.596 (±0.073) between MY and DMI, with the strongest estimates found when using the 50k subset. However, for the other two subsets, correlations were only slightly lower, highlighting the substantial relevance of the genomic regions they contain for MY, DMI, BW and EB. Overall, the results support the hypothesis that the analyzed subsets of SelS and mQTL harbor causal mutations that substantially contribute to the genetic variation of the investigated traits. In a next step, a direct health trait (e.g. mastitis) will be incorporated into the analyses in order to investigate its relationship with the traits already examined, which could provide valuable insights for improving animal health and breeding strategies.

Genetic parameters of residual carbon dioxide as a feed efficiency indicator in Fleckvieh cows

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Enhancing feed efficiency is a key goal in livestock breeding for economic and environmental reasons. However, routine recording of feed intake, which is required to derive feed efficiency traits, is unavailable for Fleckvieh (dual-purpose Simmental) cows. Residual carbon dioxide (RCO2) may be a suitable proxy, since CO2 production reflects metabolic heat production, which is linked to feed intake and metabolic rate. The objectives of this study were therefore to (1) characterize phenotypic patterns of RCO2 across commercial farms, and (2) estimate the heritability and genetic correlations of RCO2 and related traits. The dataset comprises CO2 data collected from 14 Austrian commercial farms using a GreenFeed system for a period of at least six weeks, alongside with daily milk yield (MY), weekly milk samples, and daily body weight (BW). It included 722 Fleckvieh cows, with 62,756 GreenFeed records, 26,688 MY records, and 30,759 BW records, aggregated to 1,836 weekly averages from 427 cows with complete data. The five-generation pedigree included 20,056 animals. RCO2 was defined as the residual from a linear model regressing weekly CO2 on energy-corrected milk (ECM), metabolic BW, and BW change, including test week as a random effect. The differences between farms for CO2 and RCO2 were first descriptively analyzed and then tested using Welch's ANOVA, followed by Games-Howell post-hoc comparisons. For the genetic analyses, a multiple-trait repeatability animal model was used to estimate the genetic parameters of ECM, BW, CO2, and RCO2. The model accounted for the fixed effects of herd, parity, and days in milk, as well as the random effects of herd-trial-test-week, permanent environment, and additive genetic effects of the animal. The mean values of CO2 and RCO2 differed significantly between farms, ranging from 12,228 to 15,476 g/day and -1,360 to 561 g/day, respectively. Additionally, substantial variation was observed within farms. The heritabilities of CO2 and RCO2 were low to moderate, and the two traits were strongly genetically correlated. As expected, CO2 showed stronger genetic correlations with ECM and BW than RCO2. In conclusion, these findings provide initial insights into RCO2 derived from on-farm data and its genetic properties for Fleckvieh cattle. Validation against station data with measured feed intake is a critical next step.

The impact of selection for methane emissions in dairy cattle on herds with different sizes

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Livestock farming contributes 16% of methane emissions worldwide (Vettoretti, 2024). Moreover, it has been shown that production traits and methane, a greenhouse gas, emissions were positively correlated in dairy cattle ($\rho = 0.2$). Thus, including new traits, such as methane emissions, in selection for dairy cattle is a solution to control the impact of livestock production on global warming and climate change (Bačėninaitė et al. 2022). The ISU (Unified Selection Index) defines the selection objectives of a breed. In particular, the ISU indicates the traits under selection and their selection intensity, determined by their economic value, heritability and genetic correlations with other traits. For example, in the Holstein dairy breed, the 2024's French ISU is mostly defined by milk production, fertility, morphology and health. No environmental considerations are included. (Prim'Holstein France 2024) Although some countries, such as Denmark, are already including methane emissions into their selection program (Hafsa 2024; VikingGenetics 2025), one of the main obstacles to adding methane emissions into the dairy cattle's ISU is the lack of objective economic values attributed to them. Moreover, milk production and methane emissions are positively correlated, leading to a potential decrease of the production if methane emissions are selected against. The imminent demand of an ISU including methane emission drives the need to understand the impact of such new indexes, not only at a population level but also on each farmers' context. Particularly, depending on the herd size of each farm, the economic impact of including methane emission in the ISU will vary and will depend on total milk production relative to maintenance costs. For example, a loss of 10% milk yield for a farm with 10 cows will have a much greater economic impact than for one with 150 animals, where maintenance costs per kg of milk tend to be lower.By simulating a dairy cattle breeding program with four traits representing production, fertility, health and methane emissions, we aimed to study the impact of adding methane emission in the selection index on the genetic progress of all traits, and determine the optimal selection strategy based on the herd size. Traits were simulated with genetic parameters similar to those of a French Holstein population, in particular with a genetic correlation between production and methane emission equal to 0.2. Different weights for the four traits were tested, from the current ISU with no weight on methane emission, to strong selection against it. Cows were separated into 200 farms of different sizes (5, 15, 35, 75 or 145 cows) with a common bull nucleus for selection. We evolved the populations over 10 generations, comparing the genetic progress and the trends in genetic variances and correlations for the different herd sizes. Preliminary results have been obtained, showing coherence with the initial chosen parameters. We observed an increase of the methane emissions in the cases of the ISU without methane emissions, similar for all farms. On the contrary, genetic variances of small farms compared to large ones were lower, but with higher standard errors. These results thus show that herd size plays a role on the consequences of selection, and should be considered when defining breeding goals. This study was carried out as part of the project OPTI-HERD, which simulates farming systems, taking into account adaptation and mitigation of climate change.

Genetic mitigation of methane emissions in Nordic Red dairy cattle through international data integration and joint genomic evaluation

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Enteric methane (CH_4) emissions from dairy cattle represent a significant source of greenhouse gases. Genetic selection can serve as a mitigation strategy, provided there are extensive, well-connected reference populations and standardized phenotypes across different recording methods. While methane breeding values have been developed for several Holstein populations, their adoption within Red Dairy Cattle (RDC) breeds remains limited. The purpose of this project is to promote international phenotyping and harmonized data integration, enabling reliable genomic predictions of methane emissions, and supporting their implementation in breeding programs for RDC populations. The Red Dairy Project, part of the Global Methane Genetics initiative, aims to accelerate methane phenotyping in RDC while integrating data from multiple countries and recording systems. Methane measurements are collected using sniffers and GreenFeed units, with overlapping data sets obtained from selected herds. Fourteen sniffers are being installed across Denmark, Finland, Norway, and Sweden following standardized protocols and harmonized editing pipelines. Traits under consideration include methane production, concentration, intensity, and residual methane. Single-step genomic BLUP models will be used for genetic analyses, and data from Canada and the United Kingdom will be included to evaluate the effect of expanding the reference population and to quantify genetic connectedness. This project lays the groundwork for joint genomic studies of methane emissions in RDC breeds. By creating the largest standardized methane dataset encompassing diverse countries and recording systems, this initiative facilitates effective low-methane breeding programs within RDC and promotes international data sharing to expand global impact.

Mitigation strategies for methane emission in dairy cattle production

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Enteric methane (CH_4) in dairy cattle is produced during anaerobic rumen fermentation by methanogenic archaea and represents a major source of greenhouse gas emissions as well as a loss of dietary energy that reduces feed efficiency. Therefore, methane mitigation is an important target for improving both productivity and environmental sustainability in dairy systems. This review assessed methane mitigation strategies in dairy cattle, focusing on nutritional modification, microbiome-based interventions, and genetic selection. Nutritional modification showed the most immediate mitigation potential. Supplementation with 3-nitrooxypropanol (3-NOP) produced the strongest and most consistent reductions, lowering methane production by 32.7%, methane yield by 30.9%, and methane intensity by 32.6% at a mean inclusion rate of 70 mg/kg diet dry matter. These reductions, equivalent to nearly one-third of emissions, indicate substantial improvements in feed energy utilization and a meaningful decrease in the environmental footprint of milk production. Dietary nitrate supplementation achieved moderate reductions, decreasing methane production by at least 20.3%, methane yield by 16.4%, and methane intensity by 20.1%, although responses varied with diet composition. Tannin-based additives reduced emissions by approximately 10%, suggesting a supportive role, while essential oil blends resulted in smaller but measurable reductions (8.8% in methane production, 12.9% in methane yield, and 9.9% in methane intensity) during long-term supplementation. Microbiome-based interventions demonstrated that methane mitigation is closely linked to shifts in rumen microbial structure and function. The effect of 3-NOP was associated with reduced abundance and transcriptional activity of Methanobrevibacter spp., including downregulation of the mcrA gene central to methanogenesis. Rumen defaunation led to short-term reductions of 20–50%, highlighting the importance of protozoa–methanogen interactions, although these effects were often transient. Rumen microbiota transplantation produced smaller reductions of 5–15%, with microbial communities frequently reverting toward their original state, indicating limited long-term stability. Genetic association studies reported moderate heritability estimates (0.18–0.24) for methane production, yield, and intensity, indicating that 18–24% of variation in these traits is attributable to additive genetic effects and confirming the potential for sustained genetic progress through selection. While nutritional and microbiome-based strategies are effective during active application, genetic selection offers cumulative and permanent reductions across generations. Overall, feed-based interventions can deliver rapid reductions of up to one-third of emissions, whereas genetic selection provides a long-term, scalable strategy for sustainable methane mitigation in dairy production systems. Keywords: enteric methane; dairy cattle; genetic selection; rumen microbiome; sustainable livestock production

First measurements of methane emissions in the Manech Tête Rousse dairy sheep breed

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Livestock productions are facing several environmental, societal and economical challenges. Major environmental challenges relate to climate change, such as feed-food competition, use of arable lands and the environmental footprint. Ruminants, thanks to their ability to transform fibers into edible food can marginally contribute to the feed-food competition, but the unfavourable counterpart of the ruminal fibres' fermentation is methane emissions. Several levers are investigated to tackle methane emissions from ruminants, genetic selection being one of them. Phenotyping is the first step when it comes to implement a new trait in a breeding program. In sheep, Portable Accumulation Chambers (PAC) have been developed to enable phenotyping of methane emissions on large numbers of animals, directly in their production areas and systems. PAC have been mainly used in meat sheep for which genetic parameters have already been published for different breeds. In dairy sheep, the literature is scarce both for PAC phenotyping and for estimates of genetic parameters of methane emissions. INRAE is equipped with PAC and first measurements on dairy sheep were performed on Manech Tete Rousse (MTR) rams bred at the CDEO animal insemination centre. A total of 243 MTR rams were phenotyped in 2025. Young rams genomically preselected (1 year old) were phenotyped in December whereas older rams (with daughters) were phenotyped in November. All these rams have genomic estimated breeding values (GEBVs) for milk traits (milk, protein and fat yields, fat and protein contents), somatic cells and udder morphology, as well as a production index combining yield and content (milk_index) and a total merit index combining all the above selected traits (TMI). PAC measurements consist in 3 gas traits (CH4, CO2 and O2) in g/d, from which 2 additional traits are calculated: (CH4+CO2) considered as a proxy of feed intake and methane yield calculated as CH4 divided by this proxy (ie (CH4+CO2)). All rams had body weight recorded during the week of the PAC measurements. The objective of this work was to describe gas traits and to estimate correlations between these traits and selection indices. On average, rams emitted 22.97 ± 6.98 g CH4/d, 1943 ± 839 g CO2/d and consumed 1174 ± 355 g O2/d. Old rams emitted more methane than young rams (21.29 g/d and 25.35 g/d, respectively), in line with the significant difference in body weights (49.47 kg and 67.71 kg, respectively). Similarly, older rams emitted more CO2 and consumed more O2 than young rams. However, methane yield was not significantly different between old and young rams. These phenotypic values are consistent with those in the literature, although most studies have been conducted on meat sheep breeds. No significant correlation was estimated between methane traits and GEBVs. Body weight was significantly correlated with selection indices (milk_index and TMI), but this was due to the known bias with genomic evaluations where young animals (which are also lighter than older animals) are overestimated. This significant correlation disappeared when the age of the animals was considered. Similarly, some correlations between CO2 and O2 were significantly negative with selection indices, but turned to be equal to zero when the age of the animals was considered.

Development of QuickFlux for measuring enteric methane and carbon dioxide fluxes from dairy cattle and their association with dry matter intake

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In dairy production systems, enteric methane (CH4) is a major contributor of greenhouse gas emissions. In addition to its environmental impact, enteric CH4 represents a loss of gross energy that could otherwise be directed toward production. Moreover, CH4 and carbon dioxide (CO2) have been explored as predictors of intake and feed efficiency. The objective of this study was to evaluate the association between gas fluxes, measured using a novel technology (QuickFlux, C-Lock Inc.), and dry mater intake (DMI) in dairy cows. QuickFlux was developed to quickly and quantitatively measure mass flux rates of metabolic gases from the muzzle of cattle, it includes a gas-capturing assembly consisting of a collector, airflow pipe, air filter, fan that pulls air through the system at 40 L/s, and individual gas sensors. Gas mass flux rate is calculated by multiplying the airflow rate by the increase or decrease in concentration of each gas from its background and ideal gas laws are used to convert gas volume to gas mass in g/d. Data were collected from 76 multiparous lactating Holstein cows at California's Central Valley (DairyExperts Inc., Tulare, CA). QuickFlux sampling was conducted while animals were in the milking parlor. Each cow was sampled 9 times: 7 samples were collected once weekly during the morning milking (0700-1000 h), and in the final 2 weeks, samples were also collected during the afternoon milking (1800-2000 h) on the same day. Cows were fed a common diet and housed in a free-stall barn with access to GreenFeed. QuickFlux measurements (CH4 and CO2; g/d) were collected individually and the duration of each measurement was 5 min. Daily milk yield (MY) and DMI, lactation number, and days in milk (DIM) were recorded. Partial regression coefficients and coefficients of determination (adjusted R^2) for DMI were compared between QuickFlux and GreenFeed using six multiple linear regression models. All models included DIM and lactation (Lac), both with 3 levels. Model 1 included DIM, Lac, MY, CH4, and CO2; Model 2 included DIM, Lac, MY, and CH4; Model 3 included DIM, Lac, MY, and CO2; Model 4 included DIM, Lac, CH4, and CO2; Model 5 included DIM, Lac, and CH4; and Model 6 included DIM, Lac, and CO2. Variables were averaged per cow. Adjusted R_2 values were similar between QuickFlux and GreenFeed for all models: 0.68 and 0.66 for Model 1; 0.62 and 0.59 for Model 2; 0.66 and 0.67 for Model 3; 0.54 and 0.49 Model for 4; 0.44 and 0.36 for Model 5; 0.49 and 0.48 for Model 6. Partial regression coefficients did not differ between QuickFlux and GreenFeed. CO2 slopes were significant ($P < 0.01$) for all models and were approximately 0.001 g/d. CH4 slopes were not significant in models that included CO2 (Models 1 and 4); in Models 2 and 5, the slopes were 0.02 and 0.03 g/d, respectively. Our results showed that predicting DMI using CH4 and CO2 fluxes may lead to similar results whether they are measured with QuickFlux or GreenFeed, suggesting that QuickFlux is a feasible technology for measuring gas fluxes in dairy cows, with strong potential for predicting DMI and feed efficiency. Keywords: GreenFeed, feed efficiency, phenotyping

Genetic and epigenetic hotspots in the Nelore cattle genome associated with residual methane emission

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Methane emissions from cattle are a major contributor to greenhouse gas emissions. Understanding its biological regulation is essential for sustainable livestock systems. Herein, we identified genetic and epigenetic regulatory hotspots associated with residual methane emission (RME) in Nelore cattle by integrating genetic and epigenetic data from rumen tissue. We selected contrasting sample groups for low (LRME, N=4) and high (HRME, N=4) residual methane emission for ChIP-Seq and ATAC-Seq assays from a population of 26 animals with available RNA-Seq and residual methane emission data. Differential expression analysis identified 21 differentially expressed genes (DEGs), e.g., ADIPOQ, LIPE, PLIN1, CIDEC, THRSP, and TRARG1 associated with lipid metabolism and others linked to immune response and intracellular signaling pathways. Epigenomic analyses revealed eight differentially bound regions (DBRs) for H3K4me1, 506 for H3K4me3, 10 for H3K37ac and 12 for CTCF, and 11 differentially accessible regions (DARs). Footprinting analysis of ATAC-Seq data identified 83 transcription factors (TFs) with differential binding patterns between groups, resulting in thousands of specific TF binding sites (TFBS) per group. Integration of epigenomic and genetic variation data identified eight DEGs with TFBS unique to one group, including SMOC1 and MYOC, which also harbor SNPs with significantly different allele frequencies between LRME and HRME. Additionally, 13 regions of 13 different genes showed overlapping DBRs across two histone modifications, some of which also contained group-specific TFBS. These integrated signals defined 21 candidate hotspots, being eight genes and 13 epigenomic regions, associated with residual methane emission. Functional annotation suggests that these hotspots are involved in energy metabolism, immune modulation, and cellular signaling processes in the rumen tissue. Our results provide a view of the regulatory mechanisms underlying methane emission in cattle, with coordinated genetic and epigenetic control in the rumen. The identified hotspots are candidate targets for future studies and potential biomarkers for genetic and epigenetic selection aimed at reducing methane emissions in cattle. Keywords: RNA-Seq, histone modification, methane.

Estimation of genetic parameters of resilience indicators from feeding behavior in the Iberian pig

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Animal resilience refers to the capacity of an animal to maintain stable performance under environmental or health-related challenges and to recover rapidly to its original or optimal state. Despite its growing relevance in breeding programs, there is still no consensus on the most appropriate indicator to quantify resilience. The objective of this study was to evaluate the genetic potential of three resilience indicators derived from feeding behavior: (a) the natural logarithm of the mean squared error (lnMSE), (b) skewness (Skew), and (c) first-order autocorrelation (Lag1). For each animal, a simple linear regression of the trait was fitted. The residuals, representing deviations from expected performance, were used to compute lnMSE, Skew, and Lag1. These indicators capture complementary dimensions of resilience: lnMSE reflects the overall magnitude of deviations, Skew identifies asymmetric perturbations, and Lag1 quantifies the temporal persistence of deviations. Indicators were computed from records of daily feed intake (DFI), time per visit to the feeder (TPV), and number of visits per day (NVD) from 1,573 purebred Iberian males monitored between 80 and 180 days of age at a commercial testing station in Spain (2020–2025). The pedigree included 2,899 animals. Variance components, heritabilities, and genetic correlations were estimated using bivariate animal models through Bayesian analysis with a Gibbs sampler. Heritability estimates for lnMSE were 0.305, 0.334, and 0.233 for DFI, TPV, and NVD, respectively, and were higher than those obtained for Skew and Lag1. For Skew, heritabilities were 0.167, 0.082, and 0.161, while for Lag1 they were 0.175, 0.095, and 0.147. Genetic correlations showed heterogeneous and in some cases antagonistic patterns: Lag1 indicators generally showed positive correlations with each other, lnMSE exhibited more neutral to slightly negative correlations, while Skew displayed the most divergent correlations (0.538 to −0.836). Some were clearly positive (>0.70), such as lnMSE for TPV and Skew for NVD (0.912) or Skew for TPV and lnMSE for NVD (0.798). In contrast, others were strongly negative, such as Skew and lnMSE for TPV (-0.871) or Skew for DFI and Lag1 for TPV (−0.820). Given the low heritabilities and moderate sample size, these correlations should be interpreted with caution. Overall, the results indicate that these indicators exhibit heritabilities high enough to support breeding strategies aimed at improving resilience. However, the variability in genetic correlations suggests that each indicator captures distinct aspects, highlighting the need to refine or combine metrics for a more comprehensive characterization of resilience. The antagonistic relationships between some trait pairs may represent genuine opposing genetic mechanisms but could also be influenced by statistical uncertainty due to low heritabilities, underscoring the need for larger datasets and further validation before drawing definitive conclusions about the genetic architecture of resilience traits or making recommendations for indicator selection in breeding programs.

Absence of trade-offs between productive traits and immune competence in Dohne Merino

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A potential antagonism between productive and efficiency traits and the ability of livestock to develop an adequate immune response has been proposed in ruminant production systems. Therefore, the aim of this study was to evaluate productive performance and feed efficiency in Dohne Merino ewe-lambs with contrasting immune competence measured at weaning. The performance of forty-four Dohne Merino ewe-lambs (2024 cohort) was evaluated during their first 10 months of life. Animals were spring-born (August-September), and their ability to mount cell-mediated (Cell-IR) and humoral (Ab-IR) immune responses was assessed at weaning. Ewe-lambs were vaccinated subcutaneously at marking (7-weeks of age) and weaning (14-weeks of age) with 1.0 mL of a clostridial vaccine (Clostrimax T®). On day 12 post-weaning, two intradermal injections (100 µL each) were administered in the inguinal region: vaccine antigen and a sterile saline control (0.9% NaCl), using a 30G needle. Skinfold thickness at each injection site was measured in triplicate immediately before injection and again 48 h later (day 14). The magnitude of the cell-mediated immune response was calculated as the log ratio of post-injection skin thickness at the vaccine site relative to the control site, adjusting for baseline measurements. Humoral immunity was assessed by measuring serum anti-tetanus toxoid IgG1 concentration in blood samples collected on day 14 post-weaning. Antigen-specific IgG1 was quantified by indirect ELISA, with samples and controls analysed in quadruplicate and quality-controlled using coefficient of variation thresholds. Residual feed intake, feed intake, methane emissions, animal growth, rib eye area and backfat thickness were evaluated immediately after immune competence assessment during a 56-day feed efficiency test. Subsequently, resistance to gastrointestinal nematode infestation was assessed in autumn. First shearing was performed when animals were 10 months old, at which time fibre diameter, fleece weight and body weight were recorded. Immune competence (IC-Comb) was defined as a composite phenotype based on the combined ranking of antibody-mediated (Ab-IR) and cell-mediated (Cell-IR) immune responses for each hogget. Rankings were derived from model residuals (observed minus predicted) obtained separately for Ab-IR and Cell-IR, using models that included age of dam and birth type as fixed effects and ewe-lamb age at measurement as a covariate. Residuals for each trait were standardised by dividing by their respective standard deviation, and IC-Comb was calculated as the mean of the standardised Ab-IR and Cell-IR residuals. The effect of ewe-lamb IC-Comb group (25% low, 50% medium and 25% high) on production, efficiency and health traits was analysed using a generalized linear model. Body weight at different ages, fleece weight, wool fibre diameter, rib eye area, backfat thickness, feed efficiency, methane emissions, feed intake and resistance to gastrointestinal parasites did not differ ($P > 0.05$) among ewe-lambs from different IC-Comb groups. These preliminary results indicate an absence of trade-offs between immune competence and relevant productive, efficiency and health traits in extensive grazing systems. This lack of antagonism supports the potential inclusion of immune competence as a novel phenotype in national breeding programs aimed at selecting sheep with an enhanced ability to develop adequate immune responses. However, because these results are not fully consistent with previous research, a larger database including multiple years of evaluation is required to confirm these initial findings.

Activity-based resilience indicators and their relationship with health in dairy cows

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Breeding objectives for dairy cows have been shifting from a production focus to more balanced breeding goals that also include aspects related to resilience. Resilience – the ability of animals to cope with short-term disruptions, including those caused by pathogens, through response and rapid recovery to their original state – is often assessed based on deviations in production traits. However, changes in production traits might be a later (i.e., 'slower') indicator of stressors occurring than behaviour-based indicators and are not available for the whole life span (e.g. before calving). Therefore, this study examines activity-based indicators in dairy cows and assesses whether these are informative for resilience. Activity data were collected on a total of 229 dairy cows from five different farms in the Netherlands. These cows were fitted with leg accelerometers, that recorded data on the number of steps taken (nStep), number of minutes per hour in which a cow walked (mWalk), minutes standing (mSta), the sum of mWalk and mStand (mSsta), minutes lying (mLie), number of times getting up (nUp), as well as neck accelerometers recording the neck activity (NeckAct). Moreover, the collected data on cows' positions in the barn over time were used to calculate distances moved by individual cows (Dist), based on raw data. The agreement between the different locomotion activity indicators (nStep, mWalk and Dist) was examined. Health data – available from previous research – consisted of clinical assessment and blood values (measured in week 1-5 after calving), which provided a total deficit score that could be separated into health components such as lameness, inflammations and metabolic aspects. The natural logarithm of variance (LnVar), the autocorrelation and skewness were calculated as potential resilience indicators, for each of the different activity measurements from the accelerometers using the R package EMOTIONS based on curves fitted with an ensemble model. The LnVar was corrected by the mean of the activity curve to account for the general activity level of the specific cow. The correlation between the activity-based indicators from the accelerometers and the different health scores was assessed. The analyses indicated a high agreement between nStep and mWalk ($r = 0.99$) as well as the LnVar calculated based on these traits, while the agreement between nStep and Dist was low to moderate. The highest correlation of an activity-based indicator with health scores was observed for LnVar corrected by the mean. The trait mSsta showed the highest positive correlations ($r = 0.51$) with health scores, albeit just slightly higher than for mSta ($r = 0.45$). In contrast, the correlations of the health scores with the autocorrelation and skewness were low or even negative for all considered traits, and for all other considered traits than mSsta and mSta the LnVar also showed low correlations with health scores. These results showcase the potential of activity-based patterns as resilience indicator traits to capture resilience to the transition phase, which cannot be captured using production-based indicators that may show a delayed response. The time a cow stands or walks seems to be the most promising measurement to build a resilience indicator trait. Such accelerometer-based indicators allow for large scale phenotyping and may have potential to be included in breeding programs to improve cattle resilience. This work was co-funded by the European Union's Horizon Europe Project 101136346 EUPAHW.

Circadian rhythm of feed intake and heritability estimates in Romane sheep divergently selected for feed efficiency

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Selection for improved feed efficiency is widely implemented across livestock systems to reduce feed consumption and nutrient excretion but its effects on feeding behaviour remain poorly understood. In ruminants, less efficient animals tend to show stronger diurnal feed intake than more efficient ones. However, the relationship between feed efficiency and day-to-day consistency of feed intake, as well as the genetic determinism of these trait, remains unexplored. Therefore, this study aimed (i) to assess the ability of Romane sheep to exhibit a 24-h periodicity of feed intake according to sex and genetic line for feed efficiency using automatic feeder data and (ii) to estimate heritability of circadian traits. Between 2022 and 2025, about 650 Romane sheep (363 males and 284 females) were fed ad libitum (concentrate or forage) at INRAE P3R (https://doi.org/10.15454/1.5483259352597417E12). Each individual visit to the feeders (whatever the feed delivered) was recorded with intake, duration and hour. Studied animals belonged to two divergent lines selected for residual feed intake (RFI). The efficient line (RFI-) included 169 males and 113 females and the less efficient line (RFI+) 194 males and 171 females. In males, feed intake per visit was recorded in spring for 40 days between 3 and 5 months of age on a 100% concentrate diet. They were subsequently phenotyped in summer, between 6 and 8 months of age, for 40 additional days under a mixed forage diet. Females, sisters of the phenotyped males, were recorded only during these 40 days under forage conditions. A time-frequency approach (wavelet) was applied to hourly feed intake to detect 24-h periodicity. Wavelet analysis is widely used to identify rhythmic activity patterns in mammals by quantifying similarity between the observed signal and a sinusoidal function evaluated over periods and shifted along the time series. The percentage of days with detectable 24-h periodicity (hereafter circadian trait) was calculated for each individual over four periods: P1 and P2 corresponded to the first and second 20-day concentrate phases; P3 and P4 to the respective forage phases. Heritability of circadian trait in each period was estimated using a univariate animal model with Blupf90 software. For each period, animals were assigned a binary variable: 0 = no 24-h periodicity; 1 = at least one day with a 24-h periodicity. A Chi^2 test compared frequencies between RFI lines (R4.2.1). In males, a 24-hour periodicity was observed on fewer than 2% of days in P1 and on approximately 10% of days in P2. The percentage increased with age, with a 24-hour periodicity detected on 16% and 21% of days during P3 and P4, respectively. Heritability estimates of these traits were near 0 in P1, 0.24 ± 0.11 in P2, and reached to 0.19 ± 0.10 in P3 and 0.31 ± 0.11 in P4. In females, percentage of days with a circadian rhythm also increased from 17% to 20% in P3 and P4, respectively. Heritability was 0.41 ± 0.13 in P3 and 0.40 ± 0.14 in P4. Comparison between RFI lines showed sex-dependent trends in circadian feed intake rhythms. Among males, the proportion of animals showing a circadian feed intake rhythm did not differ between RFI lines, regardless of diet. In contrast, during the forage phase in females, a significantly greater proportion of RFI+ (46%) exhibited a circadian rhythm compared with RFI- (31%). This study showed that circadian feed intake patterns develop progressively with age in Romane sheep and that this trait is partly genetically-driven. Selection for feed efficiency had little effect on the circadian feed intake patterns.

Heritabilities of Nitrogen Use Efficiency and Nitrogen Loss for Norwegian Red cows vary over the course of lactation

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The environmental and economic pressures to reduce nitrogen surplus in livestock systems are increasing. It has already been documented that there is genetic variability in nitrogen use efficiency (NUE), but how phenotypic variance is partitioned into its causal components across the lactation cycle has not been previously explored in Norwegian Red cows (NRF). The objective of this study was to explore how genetic variance for NUE and nitrogen loss (NL) changes throughout lactation and to examine their genetic correlation. The data used for this study were collected from 1932 NRF cows across 16 herds located in different regions of Norway. The NUE is defined as the proportion of dietary nitrogen intake that is recovered in milk nitrogen output, expressed as a percentage, whereas NL is calculated as nitrogen intake minus nitrogen output in milk, expressed as kg/day. NUE and NL were calculated as 4–7-day averages using test-day records and data from the preceding six days. Descriptive statistics showed that the mean (± SE) of NUE and NL were 30.41% (5.77) and 0.37 kg/day (0.08), respectively. The phenotypic correlation between NUE and NL was -0.46. A random regression (RR) animal model was used to examine the genetic variability in NUE and NL across the lactation cycle. The fixed effects considered were Herd-Year-Season, parity, and days in milk (DIM), modelled with a 2nd-order Legendre polynomial; for the random additive genetic effect, DIM was considered as a 2nd-order Legendre polynomial, and residual effects were considered homogeneous. Pedigree information, consisting of 4,957,544 records, was narrowed to the last 5 generations (8950 animals) to construct the additive relationship matrix. The genetic variability in both NUE and NL did indeed change throughout lactation. The heritability of NUE decreased from 0.33 (SE = 0.037) at 5 DIM to 0.21 (SE = 0.033) at 30 DIM and stayed within the range of 0.10 (SE= 0.023) to 0.22 (SE = 0.034) until 320 DIM before rising to 0.33 (SE = 0.049) at 346 DIM. The heritability of NL stayed in the range from 0.07 (SE = 0.009) to 0.17 (SE = 0.03) between 20 DIM and 300 DIM, and then abruptly increased to 0.49 (SE= 0.03) at 340 DIM. To calculate the genetic correlation (rG) between NUE and NL, a bivariate repeatability animal model with the same fixed effect as RR model and random additive genetic/permanent environmental effects was used, estimating an overall rG = -0.571 (SE = 0.137), alongside three stage specific estimates across three DIM ranges; 5–120 days (rG = -0.66 ± 0.20), 121–240 days (-0.15 ± 0.22), and 241–346 days (-0.31 ± 0.46). Overall, the heritability of NUE was moderate in early lactation, declined and remained relatively low throughout mid-lactation, then increased again toward the end of lactation, whereas NL showed low-to-moderate heritability across most lactation, with an increase in late lactation. The rG between NUE and NL was strongly negative in early lactation and remained low to moderate from mid to late lactation. The RR model provided information on changes in genetic variability across the lactation cycle. But the model was applied to a dataset with a relatively few records per lactation (4 on average). Moreover, in the early and late stages of lactation, the data included relatively few observations. These limitations likely contributed to the increase in heritability for NUE and NL at the extremes of the lactation cycle. However, genetic variability for both NUE and NL across lactation stages highlights the potential to improve these traits through selective breeding in NRF cows.

Combining mechanistic energy allocation and genetic models to explore multiperformance in dairy sheep population

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Enhancement of dairy sheep multiperformance at the population level requires simultaneous progress in production and environmental traits, such as reducing enteric methane emissions per unit of milk. Mechanistic models explicitly represent the underlying biological functions that drive these traits and their trade offs, and can be run under many nutritional environments and management regimes to explore how alternative strategies affect lifetime performance. Genetic selection is another promising lever to enhance animal multiperformance at population level. Predicted gains are typically evaluated using additive genetic models in which animal performances are simulated based on the infinitesimal model. Combining these two types of models offers a promising approach to assess the benefits of selection strategies across diverse environments and to better account for potential trade-offs among traits. Mechanistic energy allocation models predict how animals acquire and partition dietary energy into maintenance, growth, body reserves and lactation day by day across their life and under diverse feeding conditions. This allocation pattern can be represented as an individual, genetically determined strategy that is heritable and can respond to selection. In this work, the energy acquisition and allocation coefficients are modelled as quantitative genetic traits and each ewe carries additive genetic values for these coefficients, which are transmitted through the pedigree. The distribution of these coefficients in the population is dependent on their mean and their phenotypic and genetic variances, provided as model inputs. For each ewe, the mechanistic model generates daily trajectories of feed intake, methane emissions, milk yield and body weight across multiple years and nutritional environments, which are aggregated into annual phenotypes such as cumulative methane, methane intensity, total milk yield and mature body weight. We then asked whether realistic levels of genetic variation in these output traits can be generated through genetic variation in a limited number of biologically interpretable input coefficients, and what ranges of heritabilities and genetic correlations are attainable. Preliminary analyses show that genetic variation in methane, milk and body weight can be successfully simulated by assuming heritable variation in a subset of acquisition and allocation coefficients related to intake level and allocation to lactation, whereas variation in other coefficients has negligible impact on these outputs. By tuning the magnitude of genetic variation in these key coefficients, the framework is able to reproduce a broad range of heritabilities and genetic correlations between methane, milk yield and body weight. This way, the model can be calibrated with values consistent with those reported in the literature. The combined mechanistic–genetic framework thus acts as a virtual breeding platform to test alternative selection strategies before field implementation. Thereby, it supports the design of breeding programs that contribute to the multiperformance of dairy sheep systems.

The Third State of the World report on Animal Genetic Resources: first insights

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Drawing on a wide range of sources, including data on approximately 9 000 breeds reported in the Domestic Animal Diversity Information System (DAD-IS), official reports from 112 countries and 17 organizations, and contributions from more than 100 authors through thematic analyses and case studies, the Third State of the World report provides a comprehensive appraisal of the global status of animal genetic resources. Although modest progress has been made over the past decade in breed characterization and monitoring, breeding, conservation, and sustainable use, substantial challenges remain. Around 50 percent of livestock breeds are currently with unknown risk status, and over 60 percent of breeds with a known risk status are considered at risk. While animal identification is reported for more than 80 percent of cattle breeds worldwide, only about one third are subject to genetic evaluation, a proportion that has remained largely unchanged over recent decades. Country reports indicate increasing coverage of in situ conservation across major livestock species; however, ex situ conservation, particularly ex situ in vitro, continues to lag behind. Among the identified gaps, insufficient and increasingly constrained funding is consistently reported as the most critical and growing concern.

Reflections on genomic assessment of genetic variation and the future of the breed concept

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The concept of a breed is one of the defining characteristics of domestic livestock populations. The specific and specialized characteristics of breeds, and the associated within- and across-breed diversity, are prime determinants of long-term genetic gain for production and functional traits. Frequently, breeds have non-production functions and are part of the cultural heritage of ethnic groups or entire countries. Despite its paramount importance, the definition of "breed" within a livestock species remains unclear. One of the most popular definitions is "a breed is a breed if enough people say it is". While the elegant simplicity of this definition is appealing, other aspects must also be considered. With decades' worth of genomic data from a wide variety of livestock species, we can assess the genetic variation in established breeds, and in populations whose classification remains debated. From the administrative perspective, expectations and rules for breed acknowledgement can differ in various countries. While perspectives differ, certainly a verbal establishment of a breed is not enough. Consequently, genomics metrics and opinions on population characteristics of breeds are a continued source of discussion.

Establishing and scaling up breeding programmes under challenging environments

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There is a wide variety of options for the design and implementation of breeding programmes (BPs). We conducted a thematic study, investigating 17 case studies, to outline a comprehensive roadmap for advancing BPs under challenging conditions (harsh environments, difficult market access…), targeting smallholder farms in low-input and extensive systems. Community-based BPs (CBBPs) have emerged in the last two decades as ways to jointly conserve and improve local breeds. Important features of these CBBPs are i) the livestock keepers' active participation, ii) the improvement of locally adapted breeds to reduce dependence on input, and iii) the consideration of locally available infrastructure and support options. CBBPs are promoted as a tool for the economic and livelihood development of smallholder farmers in low-input systems through genetic improvement of livestock. The 17 case studies were taken from interviews and examples from a literature review, including a diversity of species (chicken, small ruminants, cattle, buffalo, pig, llamas) and products (meat, milk, eggs, wool and cashmere) from different regions (Africa, Asia, Latin America and the Caribbean). Based on the case study analysis, we addressed strategies for scaling up BPs in challenging environments, highlighting 9 main issues and practical approaches to expand their reach and effectiveness while maintaining sustainability. Breeding activities necessitate substantial investments for the various stakeholders. Case studies demonstrate that BPs will always need external inputs (technical and/or financial support) to be sustainable. This also holds in high-income countries for numerous BPs that still receive public funding. Long-term BP success depends on strong stakeholder cooperation. A prerequisite for their success is the existence of a market that can make profitable livestock breeding and products. In low-income countries, scaling up BPs is challenging due to limited infrastructure, and lack of skilled personnel, legal frameworks, and public funding. For impact, BPs must align with local development stages and available resources, while improving herd management and value chain services. Scaling up BPs is essential i) to connect dispersed CBBPs and thus, ensure systematic recording and overcome the limitations of small herd sizes in genetic evaluations; and ii) to effectively disseminate improved animals to livestock keepers in remote areas. Otherwise, only a few livestock keepers can benefit from the progress achieved. A BP can expand vertically (scaling up by increasing the number of breeding males) or horizontally (scaling out by replicating elsewhere the BP). Both strategies are feasible for small ruminants, while intensifying male use via AI is rarely cost-effective. For large ruminants, AI is often the best option due to high costs and risks of natural service. In poultry, organisations, like World Poultry Foundation and Tropical Poultry Genetics Solutions, partner with private actors to supply improved breeds, scale chick production, establish brooding units, train specialists, and empower women farmers to raise birds for meat and eggs. Case studies were too rare to decipher general rules for other species. A country with national, regional or local BPs strengthens its food security and provides job opportunities. Scientists' support is needed to assist stakeholders in their R&D actions as well as in their capacity building.

The Evolving Role of the Private Sector in Livestock Breeding

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Over the past decade, the private sector has undergone a profound transformation in its approach to the management and use of animal genetic resources (AnGR). Once characterised primarily by productivity-driven selection, breeding companies and reproduction organisations across ruminants, pigs, poultry, aquaculture and insects have broadened their breeding objectives to encompass animal health and welfare, environmental sustainability, and the long-term maintenance of genetic diversity. This shift reflects both mounting societal expectations and an evolving regulatory and policy landscape at European and global levels. Drawing on the experience of EFFAB, this presentation explores how technological advances, governance frameworks, and the broader societal context have collectively reshaped private sector engagement with AnGR since the Second State of the World Report on Animal Genetic Resources for Food and Agriculture. Evidence is drawn from private sector's data and perspectives, offering a cross-species view of progress made, persistent gaps, and emerging priorities as seen from the breeding and reproduction sector. The presentation reflects on key developments in breeding objectives, the uptake of new genomic technologies and their implications for genetic diversity management, and the regulatory environment shaping private sector operations. Looking ahead, the presentation identifies future challenges and opportunities for the private sector in the context of the Global Plan of Action for Animal Genetic Resources. It argues that realising the private sector's full contribution to sustainable AnGR management will require more inclusive governance frameworks, greater recognition of existing industry accountability mechanisms such as the Code of Good Practices for Responsible and Balanced Breeding (Code EFABAR), and stronger public-private collaboration built on mutual trust and clear data-sharing conditions.

Progress, Gaps, and Future Challenges in Animal Genetic Resources Management in Europe from a National Coordinator Perspective

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Since publication of the Second Report on the State of the World's Animal Genetic Resources, AnGR management has shifted from largely descriptive assessments to more strategic, policy-oriented approaches. Major advances have been made in characterizing and monitoring livestock diversity through improved databases, phenotypic and genomic tools, and more systematic reporting. New EU legislation has embedded genetic resources more firmly in agricultural policy, raising visibility and responsibility at national level and increasing demand for evidence-based prioritization of at-risk breeds. A key milestone is the European Union Reference Centre for Endangered Animal Breeds (EURC-EAB), which provides scientific and technical support, promotes harmonization, strengthens cooperation among Member States, and reinforces the role of breed societies and the breeding industry in sustainable breeding programmes.Regional and international coordination has intensified. In Europe, the European Regional Focal Point for Animal Genetic Resources (ERFP) has strengthened collaboration, enabled knowledge exchange, and advanced a common AnGR Strategy for Europe, supporting more coordinated in situ and ex situ action. Expansion of the European Gene Bank Network for Animal Genetic Resources (EU-GENA) has further consolidated ex situ conservation, increasingly viewed as core risk management rather than a complementary measure. In parallel, reporting and coordination mechanisms are increasingly linked to wider biodiversity and rural-development agendas. Transboundary-breed management now requires closer alignment among countries, while small populations remain vulnerable—especially to infectious disease outbreaks—underscoring the need to reinforce gene banks and establish clear emergency procedures.Important gaps persist. Many local breeds remain endangered because of limited economic viability, weak incentives for diversified products, and underdeveloped market valorization mechanisms. Data gaps still constrain assessment of genetic diversity and adaptive potential. Although genomic technologies offer opportunities, uptake is uneven and often limited by resources and capacity; capacity building and practical decision-support tools are needed to translate genomics into routine breeding and conservation action. Ensuring effective stakeholder coordination and alignment with European and global frameworks remains challenging.Looking ahead, the National Coordinator's role is becoming more complex as climate change, disease risks, evolving production systems, and regulatory developments demand integrated, forward-looking action. Stronger links between conservation and sustainable use—including ecosystem management principles and recognition of ecosystem services—will help ensure AnGR support environmental resilience and human well-being. Priorities include digitalizing data systems and enabling rapid exchange, sustaining long-term gene bank infrastructure and operational capacity, strengthening cross-border cooperation, and improving emergency preparedness. Ultimately, future AnGR management will depend not only on technology, but also on coordination, coherent policy implementation, sustained investment, and efficient data sharing across sectors and countries.

European resilient livestock farming in a continuously changing world - Welcome & introduction

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European livestock farming is going through a period of profound change. Geopolitical tensions and global trade disputes have disturbed feed supplies and reshaped market dynamics. At the same time, societal scrutiny over environmental impact, animal welfare and health is challenging the sector's legitimacy – particularly in Western Europe. Nevertheless, the livestock sector continues to play a vital role in Europe's food and nutrition security. It contributes to circular food systems, supports rural economies and provides essential nutrients. As pressures mount, the sector must innovate and engage with policy and society to secure its place in a more sustainable and resilient food future. How can Europe's livestock sector redefine its role and earn public trust while delivering sustainability and food security in an increasingly fragmented world? Most of the focus on livestock and climate change has been on emissions of greenhouse gases and opportunities to mitigate them. However, the impact of climate change on livestock agriculture is becoming increasingly important as extreme weather events become more frequent: temperature rise is approaching the 1.5 degree set in the Paris Agreement, longer periods of drought or intense rain are increasingly frequent too. In addition, EU livestock production is challenged due to the global situation as such, as e.g. feed prices may be highly influenced by the price and/or availability of the raw materials. One example is the situation experienced in Ukraine with the limited exportation of barley/wheat, and a similar situation could be experienced with the USA in relation to the export of e.g. soy protein/corn. The topic will address consequences of several factors in building resilient livestock systems, including land and water shortage, extreme heat for livestock farming and related operations, feed and food security/safety as well as lack of farmers to ensure future food production, social demands and how to minimize these impacts. Water and land requirement and resilience of livestock farming to a changing environment such as fodder availability, feed additives supply, capacity to adapt and cope with emerging diseases and other critical situations are already challenges faced by the farmers, and may be even further sharpen in the near future. The use of land and water in livestock production is under increasing scrutiny and the extreme weather events are dramatically affecting efficiency, livability and animal health, likely leading to displacements of some from farming areas and replaced by more resilient genetics and breeds. The uncertainty of climatic conditions is already dictating several changes in management practices, affecting more pronouncedly outdoor livestock systems (e.g. based on grazing areas), but also, and indirectly by influencing feed production (fodder and/or cereals, legumes, etc.), and putting systems sustainability at risk. The dramatic decrease and uncertainty on farmer generational renewal is a real problem and places future animal farming and livestock production in the EU in a difficult situation. This implicates higher food insecurity in the EU. The theme also intends to explore the implications for the upstream and downstream sectors, such as animal feeding and nutrition, breeding and medicines, food processors, etc., involving all sectors including industry (milk processors, slaughterhouses, feed companies).

The New Era is Now: Sustainable Livestock Through Global Collaboration

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Global dynamics, particularly population growth, rising incomes and rapid urbanization, have contributed to an increased demand for animal protein, making the livestock sector one of the fastest growing agricultural sectors. This means that the livestock sector is central to agrifood system transformation and plays a key role in rural development through economic growth, poverty reduction and its contribution to better livelihoods. Livestock provides food, fibre and a wide range of animal products, as well as draught power, and in most developing countries livestock provides smallholder farmers with a substantial proportion of their income. By serving as productive assets and sources of household nutrition, livestock can offer a pathway to poverty reduction and economic well-being, enhancing the resilience and adaptability of rural communities. Although the livestock sector presents numerous opportunities, it also faces significant challenges. If not properly managed, the rapid growth in the livestock sector can increasingly threaten natural resources, such as water, land and soil. The livestock sector is under scrutiny for the greenhouse gas emissions it generates, contributing to climate change. Livestock production can also impact biodiversity through expansion of the sector, changes in land use and deforestation, contributing to the loss of natural habitats and the fragmentation of ecosystems. Drawing on FAO's work across diverse regional contexts, this presentation provides a global overview of the main opportunities and challenges facing livestock systems today. It places particular attention on the role of youth, highlighting that as rural populations age and fewer young people enter the sector, attracting, empowering, and retaining the next generation of livestock keepers becomes essential for ensuring innovation, resilience, and the long-term sustainability of livestock systems.

Navigating the Future of Animal Protein in a Changing Global Landscape

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Global demand for animal protein is expected to grow over the coming decade, driven by population growth and rising income levels. This expansion will be concentrated primarily in Asia, Africa, and the Middle East. In contrast, Europe will experience slow income growth alongside a stable or slightly declining population, resulting in softening demand for animal protein. Europe's competitiveness in global markets will come under increasing pressure as production costs rise, largely due to tighter environmental and social regulations. Traditional volume-driven, export-focused business models will become less viable, creating the need for new value-creating approaches. To remain successful, animal protein companies will need to shift from prioritizing volume to delivering higher value and strengthening their focus on European consumers. This transition will be complicated by declining animal numbers as environmental constraints tighten, greater volatility stemming from geopolitical tensions, and continued dependence on global markets for key inputs. These pressures will accelerate industry consolidation. In such a challenging environment, innovation and rapid adoption of new technologies across animal protein supply chains will become more critical than ever.

Ensuring EU feed security and safety in a rapidly changing global environment

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In an increasingly volatile geopolitical, economic and environmental context, ensuring the security and safety of feed supplies has become a strategic priority for the European Union. The feed sector stands at the heart of the agri-food chain, playing a critical role in safeguarding animal health, supporting sustainable livestock production and contributing to food security for European citizens. Against this backdrop, FEFANA and FEFAC, as the two leading trade associations of the EU feed chain, jointly present an in-depth analysis of the challenges and opportunities shaping EU feed security and safety in a rapidly changing global environment. The presentation will explore the multifaceted pressures currently affecting global feed supply chains, including geopolitical instability, climate change, trade disruptions, evolving regulatory frameworks, and increasing sustainability expectations. These factors have heightened risks related to the availability, affordability and quality of feed, while simultaneously placing greater demands on safety and environmental performance. The COVID-19 pandemic and ongoing geopolitical tensions have further exposed vulnerabilities within global logistics networks, underscoring the urgent need for resilience, diversification and strategic autonomy. FEFANA and FEFAC will outline how the European feed sector is responding to these challenges through innovation and close collaboration across the supply chain. Key topics include strengthening feed safety systems, improving risk assessment and crisis preparedness, and fostering responsible sourcing strategies. Particular attention will be given to the role of feed additives, technological innovation and digitalisation in improving feed efficiency, animal health and environmental outcomes, while reinforcing food and feed safety standards. The presentation will also examine the implications of EU policy initiatives and evolving trade and sustainability regulations, on feed security and competitiveness. While ambitious sustainability goals offer opportunities for innovation and transformation, they must be aligned with realistic implementation pathways that preserve supply stability, economic viability and international competitiveness. FEFANA and FEFAC advocate for coherent, science-based and globally aligned regulatory frameworks that support both safety and sustainability without undermining the resilience of the feed sector. Furthermore, the presentation will highlight the strategic importance of international trade and partnerships in securing diversified and reliable feed ingredient supplies. It will emphasize the need for open, rules-based trade, regulatory cooperation, and alignment of safety standards to maintain access to global markets and mitigate supply risks. At the same time, opportunities to enhance EU protein self-sufficiency, circular economy approaches, and alternative feed resources will be explored as complementary strategies to strengthen long-term resilience. By bringing together the perspectives of feed additive producers and compound feed manufacturers, this joint presentation will deliver a comprehensive and forward-looking vision for ensuring EU feed security and safety. It will provide policymakers, stakeholders and industry leaders with concrete recommendations to build a resilient, innovative and sustainable feed system capable of supporting Europe's food security ambitions in an increasingly complex and uncertain global environment.

Exploring the Missing Middle: From Polarization to Systemic Responsibility in Europe's Protein Transition

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Europe faces a striking paradox: it is a net exporter of animal proteins, while its internal consumption levels largely exceed nutritional recommendations. In this context, the protein transition is often framed in stark and simplified terms. One hypothetical response would be to advocate for a drastic and immediate reduction in livestock production and consumption—full stop—as if complexity could be resolved through a single corrective measure. This scenario is not presented as a realistic or desirable policy option, but as a heuristic extreme that exposes the limits of reductionist thinking. At the opposite end, a business-as-usual trajectory relies on incremental efficiency gains to mitigate impacts without fundamentally altering the system. Both narratives—radical contraction and incremental optimization—contain elements of truth. Yet both risk offering deceptively simple answers to a structurally complex socio-ecological challenge. This keynote argues that the debate—and much of the associated research—is trapped in a polarization that obscures a crucial analytical and political space: the "missing middle" between these extremes. On the production side, sustainability is often equated with efficiency—producing more with fewer inputs and lower emissions. On the consumption side, responsibility is largely placed on individual dietary change. What disappears in this divide is a systemic understanding of livestock systems as territorially embedded, multifunctional socio-ecological systems operating within diverse environmental, economic, and institutional contexts. Exploring the missing middle requires reframing livestock not only as a source of food commodities, but also as a provider of ecosystem services, social value, and territorial cohesion. Livestock systems contribute to landscape management, biodiversity, nutrient cycling, employment, and cultural heritage. In many inner and marginal rural areas of Europe, livestock farming represents the backbone of local economies and a key factor in preventing land abandonment and demographic decline. A uniform reductionist approach—if implemented without differentiation—would risk generating uneven territorial consequences while failing to address structural imbalances along the value chain. Adopting a systemic perspective is not only an analytical shift—it has implications for European policy dynamics. It challenges sectoral silos separating agricultural productivity, environmental protection, public health, rural development, and trade policy. It questions instruments that reward efficiency without accounting for territorial externalities and multifunctionality, and it reveals the limits of governance approaches that allocate responsibility primarily to farmers or consumers. A systemic approach instead calls for shared responsibility across the value chain and governance levels. It implies greater coherence between the Common Agricultural Policy, the Green Deal, the Farm to Fork Strategy, climate and biodiversity regulations, cohesion policy, and trade frameworks. It requires valuation mechanisms capable of recognizing ecosystem and social services alongside commodity outputs, and governance arrangements enabling coordinated trade-offs. Drawing on insights from the European projects PATHWAYS and Re-Livestock, this keynote reflects on how integrated assessment and multi-actor engagement can help overcome research polarization and inform more coherent, territorially sensitive, and socially legitimate policy pathways for Europe's protein transition.

The social and economic challenges to farmers – how can generational renewal be achieved?

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The generational transition in agriculture represents a central challenge for the future of rural areas. In addition to income volatility, rising land and lease prices, and high capital requirements, clear differences between types of farms are evident. Larger farms are often confronted with significant financial burdens and investment risks when transferring ownership, while smaller, family-run farms have only limited opportunities to invest in modern technologies, which restricts their competitiveness. At the same time, their workload is particularly high due to the large share of family labor, resulting in long working hours and limited flexibility. In addition, political uncertainty—particularly with regard to animal welfare and environmental regulations—complicates long-term investment decisions. At the same time, markets must provide viable economic perspectives; a lack of profitability in certain production sectors is already leading to exit processes. Another decisive factor is access to land, which represents a major barrier, especially for new entrants. By incorporating the perspective of a family farmer and drawing directly on the speaker's practical experience, the contribution shows that generational renewal requires differentiated solutions that take economic, social, and environmental dimensions into account. A key aspect is that agricultural farms are not only confronted with increasing requirements, but also need the perspective of being able to implement their own values. For many young people—especially newcomers to farming as well as partners from outside the agricultural sector—issues such as socio-economic sustainability, animal welfare, and biodiversity are becoming increasingly important. Financial support instruments should therefore be understood less as traditional subsidies and more as compensation for public goods that are not adequately remunerated by the market. Against this background, the question arises as to the future development pathways of agricultural farms: between 14,000-liter cows and cow-calf rearing on pasture, between climate-friendly barns and biodiversity conservation, between manager and farmer, family and business. Do viable hybrid forms emerge—or does this increase the risk of overload? In light of discussions about workload and mental health, the question also arises whether, and under what conditions, young people are willing to pursue this path and how advisory systems must be designed to support them effectively.

EU Policy change related to the vision for agriculture and food, including simplification of EU rules and bureaucracy and comprehensive EU livestock strategy

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The Commission's Vision for Agriculture and Food recognises livestock as an essential component of EU agriculture and the food value chain (https://agriculture.ec.europa.eu/overview-vision-agriculture-food/vision-agriculture-and-food_en). The sector makes a major contribution to the EU economy, sustains rural communities, and supports landscape management and environmental preservation. The EU livestock sector is currently at a crossroads and faces a complex set of challenges which vary depending on different factors (size, sector, etc.) and across Member States: declining herd numbers and farm closures, persistently low profitability and weak economic viability, growing concerns about farmers' well-being, the sector's environmental and climate footprint, intensifying trade and market competition, animal health and welfare risks, and uneven territorial impact. At the same time, these challenges are driving transformation and opening up new opportunities for strengthening the sector's competitiveness and long-term sustainability. Innovation in breeding, feed efficiency, animal health and welfare, and digital technologies are all improving productivity and reducing environmental impacts. Growing consumer demand for high-quality, sustainably produced and regionally differentiated livestock products creates added-value market opportunities. Resource efficiency and environmental and climate performance are being further improved thanks to: (i) the development of circular bioeconomy models, including better nutrient management; (ii) manure valorisation; (iii) integration with crop systems; and (iv) more being made of the link between livestock production and the maintenance of grasslands. A long-term vision is needed to ensure that the EU livestock sector is crisis resilient, globally competitive, sustainable in all dimensions and reflects Europe's territorial diversity. Against this backdrop, the Commission has included a livestock strategy with elements on animal welfare as a key initiative in its work programme for 2026 (https://commission.europa.eu/strategy-and-policy/strategy-documents/commission-work-programme/commission-work-programme-2026_en). It is intended to be delivered together with a protein plan to make the EU protein system more resilient and sustainable.

European resilient livestock farming in a continuously changing world - Panel discussion

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Panel discussion with the speakers of the morning session, with the audience.

Comparison of milk mid-infrared spectra and fatty acid-based models for predicting methane emissions

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Enteric methane (CH4) emissions were predicted from milk-derived information using data collected on 16 commercial dairy farms in Austria. Methane was recorded with the GreenFeed system, and corresponding milk samples were analyzed using mid-infrared (MIR) spectroscopy. Farms operated under comparable feeding regimes based on grass and maize silage, with varying concentrate supplementation and no grazing. The final dataset included 807 records from 604 cows. Two partial least squares regression approaches were evaluated. The first model used the milk MIR spectral information, whereas the second relied on milk composition traits, including 14 individual fatty acids (FA) along with fat%, protein% and lactose%. In addition, milk yield, parity and stage of lactation were included as predictors. Under 5-fold cross-validation, predictions based solely on MIR spectra achieved moderate accuracy ($r = 0.52$; $R^2 = 0.27$) and performed better than models relying only on FA profiles ($r = 0.42$; $R^2 = 0.18$). Expanding the FA model to include major milk components resulted in a small improvement ($r = 0.44$; $R^2 = 0.20$). When animal-related variables (milk yield, parity, lactation stage) were included, predictive performance increased markedly and reached comparable levels for both approaches (MIR: $r = 0.61$, $R^2 = 0.38$; FA: $r = 0.61$, $R^2 = 0.37$). Validation across farms using a leave-one-farm-out cross validation yielded slightly reduced prediction accuracies and highlighted between-farm variability. The highest performance in this scenario was obtained when all predictors were combined (MIR-based model: $r = 0.56$, $R^2 = 0.33$; FA-based model: $r = 0.58$, $R^2 = 0.35$). In summary, milk MIR spectra and FA composition captured only a part of the variability in CH4 emissions, suggesting that they primarily reflect the underlying metabolic status. Improving prediction accuracy will require additional predictors and advanced modeling approaches to better capture the complex biological, nutritional and environmental drivers of CH4 emissions. Additional analyses are planned, using combined data from commercial and research farms.

Phenotypic and genetic prediction of methane emissions from milk mid-infrared spectra in Norwegian Red dairy cattle

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Milk mid-infrared (MIR) spectroscopy is routinely collected in dairy recording and has been proposed as a low-cost indicator for enteric methane (CH_4) emissions. However, the robustness of MIR-based CH_4 prediction across herds remains uncertain, and phenotypic prediction accuracy alone may not reflect its value for genetic improvement because MIR spectra themselves are heritable traits. The objective of this study was therefore to evaluate both the phenotypic robustness and genetic relevance of MIR-based prediction of methane emissions in Norwegian Red dairy cattle. Methane emissions were recorded using GreenFeed systems in 14 commercial Norwegian dairy herds from 2020 to 2024. Milk MIR spectra from routine test-day recordings were matched with daily CH_4 phenotypes within ±5 days of each test day, resulting in 10,218 matched observations from 1,506 cows. Methane production was predicted from 899 MIR wavenumbers using partial least squares regression. Prediction performance was evaluated using five-fold cross-validation with cow-level blocking and leave-one-herd-out validation to assess transferability across herds. Genetic parameters for CH_4 production and MIR wavenumbers were estimated using pedigree-based animal models. Prediction accuracy was moderate when evaluated with cow-level cross-validation ($R^2 = 0.31$), but decreased substantially when validated across herds ($R^2 \approx 0.05$), indicating limited transferability of MIR-based phenotypic prediction equations under commercial conditions. Heritability estimates for MIR wavenumbers ranged from 0.00 to 0.30, demonstrating substantial additive genetic variation within the MIR spectrum. Genetic correlations between MIR wavenumbers and CH_4 production ranged from −0.41 to 0.50, suggesting that specific spectral regions capture genetic variation associated with methane emissions. Although MIR spectra showed limited ability to predict methane emissions phenotypically across herds, they contained heritable variation and exhibited moderate genetic correlations with CH_4 production. These results suggest that MIR spectra may be more useful as genetically informative indicator traits than as direct phenotypic predictors of methane emissions. Incorporating MIR-derived information into genetic evaluations could therefore contribute to breeding strategies aimed at reducing enteric methane emissions in dairy cattle. This work was supported by the Research Council of Norway (projects 309451 and 346741).

Biological validation of MIR phenotyping for intake, nitrogen efficiency and methane production along the lactation curve in dairy cows

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Dry matter intake (DMI), nitrogen use efficiency for milk production (NUE), and enteric methane (CH4) production are key traits to improve sustainability in dairy production but remain difficult to measure routinely on farms. Milk mid-infrared (MIR) spectroscopy offers a low-cost phenotyping opportunity; however, its biological validity across lactation and parity still needs to be evaluated. Data were collected at the Agroscope experimental farm in Posieux between 2015 and 2024, including milk MIR spectra, feed intake, diet composition and automated head-chamber methane measurements. NUE was calculated by dividing milk nitrogen yield by nitrogen intake. To reduce the effects of daily variation, DMI and NUE were averaged across ±2 days and CH4 across ±5 d of milk sampling. We tested prediction models including linear regression, partial least squares regression (PLSR), and neural networks (NN), and evaluated them under 4-fold cross-validation, each including different sets of features combined with milk MIR data. The best model for each trait was applied to 9,975 historical milk MIR spectra to obtain estimated trait values for DMI, NUE and CH4. Prediction accuracy was moderate (DMI: R_2=0.64; NUE: R_2=0.56; CH4: R_2=0.52), and NN consistently outperformed PLSR models ($P < 0.05$). Including MIR information improved prediction of NUE and CH4 compared with animal information alone ($P < 0.05$). To test the biological validity of the longitudinal patterns of predicted DMI, NUE and CH4 along the lactation curve and across parities, the effects of lactation stage and parity were analysed using mixed-effects models. Indeed, the predicted traits reproduced biologically expected patterns: DMI increased to mid-lactation and declined thereafter, CH_4 rose rapidly after calving and stabilised, while NUE decreased across lactation. Multiparous cows showed higher intake and methane production throughout lactation, whereas their NUE exceeded primiparous cows only in early lactation. Correlations between residuals for predicted NUE and DMI as well as NUE and CH4 were near zero, but moderate (0.44) between DMI and CH4. These results demonstrate that MIR spectroscopy captures meaningful biological signals and can provide scalable longitudinal indicators of feed efficiency and environmental impact. Milk spectra therefore represent a promising scalable phenotyping tool for individual monitoring and genetic improvement of sustainability traits.

Optimizing methane emission prediction using cattle faecal near-infrared spectroscopy across confinement and grazing systems

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As concerns regarding enteric methane emissions (eCH4) intensify, numerous studies have focused on mitigation strategies in cattle production systems. The development of effective mitigation approaches requires accurate measurement of eCH4. Faecal near-infrared spectroscopy (fNIRS)-based prediction models have gained increasing attention as a non-invasive technique that can be applied to cattle without restricting their behaviour. However, limited information is available on the optimal duration of eCH4 data collection required to optimize the performance of fNIRS-based prediction models when the GreenFeed system is applied to collecting eCH4 data. Therefore, this study aimed to determine an appropriate eCH4 data collection period under contrasting production environments (i.e., indoor and grazing systems), based on the repeatability (R) of eCH4 measurements obtained using GreenFeed and the predictive performance of the fNIRS-based model. A total of 36 suckling cows conducted under indoor (P1) and grazing (P2) conditions for 105 days each. Methane emissions were measured using two GreenFeed® units simultaneously. Repeatability of eCH4 was calculated as the ratio of the animal variances to the sum of animal variances and residual error using data collected up to 100 days prior to faecal sampling into 1-, 2-, 4-, 5-, 10-, 20-, 25-, and 50-day intervals. One spot faecal sample per animal (100 g fresh weight) was collected either during natural defecation or manually from the rectum on the end of each period. Samples were subsequently dried (72h, 60°C) and ground (1-mm sieve) prior faecal reflectance spectral acquisition between 400 to 2500 nm (0.5 nm intervals) from a FOSS DS2500 instrument. Partial least squares (PLS) regression models were developed using eCH4 emission data averaged over periods ranging from 1 to 50 days prior to faecal sampling. Cross-validation errors were then compared across models. A total of 13,324 eCH4 data were recorded in P1 (419 ± 105.0 g/day), and 5,613 data were recorded in P2 (261 ± 86.2 g/day). Based on R analysis, P1 achieved stable repeatability after more than 10 days of eCH4 data collection (R = 0.73). In contrast, P2 showed a progressive increase in R with longer data collection periods, reaching a maximum R of 0.83 after 50 days. In comparison, P1 reached this level of R (R = 0.83) after 25 days. When the PLS prediction models were evaluated, the root means square error of cross-validation (RMSECV) stabilized after 2 days in P1 (13.5%). In contrast, RMSECV in P2 decreased steadily with increasing data collection length, reaching the lowest value when 50 days of data were used (RMSECV = 10.9%); P1 reached this comparable level of prediction error (RMSECV = 11.0%) after 9 days. Taken together, the R and PLS results indicate that approximately 10 days of data collection are sufficient under indoor condition, whereas more than 50 days are required under grazing condition. In conclusion, under our experimental design, indoor systems required substantially fewer days to achieve stable R values and prediction accuracy than grazing systems. As this study was designed as a proof of concept and involved only 36 animals, further validation using a larger population across more diverse production systems of cattle is warranted.

Faecal NIRS and its ability to predict digestive methane emissions in cattle supplemented with 3-NOP and Asparagopsis taxiformis additives.

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Methane (CH4) has a high global warming potential in the atmosphere. In ruminants, CH4 is mainly produced by a specific microbial population (archaea methanogens) during the digestive process in the rumen. Current methods for quantifying individual digestive CH4 emissions are difficult to implement under commercial farming conditions. Near-infrared spectra (NIRS) can be obtained from bovine faecal samples, which are an unabsorbed product of the digestion process and a universal matrix. Since CH_4 and faeces are produced during digestion, these spectra could potentially be used to detect the use of anti-methanogenic additives (AMA) and predict digestive CH_4 emissions. This work aimed to: i) evaluate the potential of faecal NIRS to detect effects of AMA supplementation in cattle diet; ii) test existing predictive models of CH4 emissions from faecal NIRS of cattle supplemented or not with AMA. This work was based on three previous experiments (Exp.) on Holstein dairy cows (n = 43; within Exp. 1 and 2) supplemented with 3-nitrooxypropanol (3-NOP; 60 mg/kg DM), and Angus beef young bulls (n = 20; Exp. 3) supplemented with Asparagopsis taxiformis macerated oil (Bromoil; 25 mg/kg DM). Individual data on CH4 were acquired using respiration chambers (Exp. 1) or GreenFeed (GF) units (Exp. 2 and 3). One spot faecal sample from each animal (100 g fresh weight) was collected either during natural defecation or manually from the rectum during the same week as CH4 measurement. Faecal samples were dried (60 °C, 72 h) and ground (1 mm) prior to NIRS analyses (between 400 to 2500 nm; Foss DS2500 instrument). For each experiment, the faecal NIR spectra of the treatments were pairwise compared (control vs. 3-NOP in Exp. 1 and 2, or control vs. Bromoil in Exp. 3). Observed CH4 emissions (g/day) were lower on average by 32% and 23% in animals fed 3-NOP, and by 67% in animals fed the Bromoil. An independently obtained partial least squares CH4 prediction model was used to predict CH4 emissions from Exp. 1. This model was built from a database of n = 342 spectral values of cattle faeces (150 dairy, 192 beef) and associated CH4 emission data recorded using GF. The pairwise comparisons demonstrated that faecal NIRS successfully identified differences in all animals fed diets supplemented with AMA. However, the predicted CH4 values from the existing model did not align with the observed CH4 measurements (in Exp. 1). The root mean square error of prediction (RMSEP) was 174.7 g/day for the 3-NOP treatment and 75.7 g/day for the control treatment. These values were mainly explained by the bias (160.9 vs -3.9 g/day for 3-NOP and control, respectively). Specific prediction models were then developed for the 3-NOP supplemented cows in Exp. 1 and 2, reducing the root mean square errors of cross-validation to 30.7 and 21.7 g/day, respectively. For Exp. 3, model calibration is ongoing. This preliminary work highlights the potential of faecal NIRS to predict CH4 emissions from cows supplemented with AMA. Keywords: Cattle, Dairy, Beef, Near infrared spectroscopy, Enteric methane prediction, Proxies, 3-Nitrooxypropanol, Bromoform

Mid-infrared prediction and genetic analyses of serum metabolites for evaluation of metabolic health in dairy cows

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Serum metabolites are relevant indicators to both assess and prevent metabolic and nutritional disorders of dairy cows during early lactation. For instance, β-hydroxybutyrate (BHB) or non-esterified fatty acids (NEFA) are used to diagnose ketosis, glucose to assess the energy status, or aspartate aminotransferase (AST) to evaluate liver function and metabolic stress. Serum metabolites are, hence, useful traits for herd management and genetic evaluation systems aiming to improve metabolic health of dairy cows. However, routine measurement is challenging in commercial farms due to cost and labour requirements. A potential alternative is the prediction from milk mid-infrared (MIR) spectra. The aim of this study was to develop prediction equations for a range of serum metabolites using MIR spectroscopy and evaluate corresponding genetic parameters. The dataset comprised 3,293 records of different serum metabolite concentrations collected on an Austrian research farm between 2014 and 2020. Partial least squares regression analysis was applied to calibrate prediction equations, utilizing a selection of 212 MIR spectra. Prediction accuracy was evaluated based on different validation scenarios, including a leave-one-cow-out and a leave-one-year-out validation approach. Subsequently, developed prediction equations were applied on a spectral dataset collected within the routine milk recording system of Austria, resulting in 51,847 MIR-spectra-predicted serum metabolite records of 23,909 Fleckvieh dairy cows with available genotype and health diagnosis data. For estimation of heritabilities and genetic correlations univariate and bivariate repeatability animal linear mixed models were applied. In the leave-one-cow-out validation, the prediction equations for BHB, urea, and NEFA obtained the highest prediction accuracy, with R^2 values of 0.79, 0.60, and 0.54, respectively. Glucose, cholesterol, bilirubin, AST, and triglycerides showed lower, but still good, predictive performance, with R^2 values of 0.42, 0.35, 0.31, 0.22, and 0.20, respectively. Performing the leave-one-year-out validation approach, showed marginally lower prediction accuracies overall. The heritabilities of the MIR-predicted serum metabolites were moderate and ranged between 0.08 and 0.18. Genetic correlations between MIR-predicted metabolites and health diagnoses varied depending on the metabolite considered, ranging from weak to strong. The results indicate that prediction equations from milk MIR spectra present a high-throughput phenotyping approach for deriving serum metabolite concentrations with moderate to high accuracy. The MIR-predicted metabolites exhibit moderate heritabilities and therefore have the potential to serve as novel traits for genetic evaluation systems aimed at breeding healthier dairy cows.

Improving milk mid-infrared prediction of dairy cow chronic stress biomarkers using machine learning and deep learning

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Animal welfare is a major societal concern, with 91% of European citizens considering it important. Chronic stress in dairy cows affects health, reproduction, and productivity, yet no routine monitoring method exists. Mid-infrared (MIR) spectroscopy, already used during milk recording, offers a cost-free opportunity to develop such a tool. Grelet et al. (2025) predicted two chronic stress biomarkers — hair cortisol (pg/mg) and blood fructosamine (µmol/L) — from milk MIR spectra in 1,442 cows from multiple European herds, using partial least square (PLS) regression, pls-discriminant analysis (PLS-DA) and support vector machine (SVM), with limited performance (coefficient of determination; $R^2 \leq 0.20$). Building on these results, we hypothesise that the biological pathway linking chronic stress to milk composition involves non-linear relationships that linear chemometric methods cannot adequately capture. This study evaluates whether machine learning and deep learning approaches can enhance MIR-based prediction of hair cortisol and blood fructosamine on the same dataset (N = 1,442; 212 wavenumbers after first-derivative preprocessing). The original PLS, PLS-DA and SVM models were first reproduced in Python. Random Forest, Gradient Boosting and ElasticNet were then applied for quantitative prediction; Random Forest, Gradient Boosting and L1/L2-regularised logistic regression with class-imbalance strategies were used for qualitative low/high classification (83/17% ratio for cortisol). In parallel, neural network architectures were explored: multi-layer perceptrons and one-dimensional convolutional neural networks designed to capture correlations between neighbouring wavenumbers. Phenotypic covariates (days in milk, parity, milk yield, breed, hair colour) were tested as additional predictors. All approaches were compared under the same herd-based threefold cross-validation as the original study, ensuring evaluation on unseen herds. Preliminary results show contrasting patterns between the two biomarkers. For fructosamine, Random Forest improves quantitative prediction over PLS (R^2, 0.06 → 0.15; RMSE, 33.9 → 32.5 µmol/L), supporting the presence of an exploitable non-linear MIR signal. For hair cortisol, no non-linear regressor outperforms PLS ($R^2 \leq 0.05$ vs 0.08), but a class-balanced logistic model raises the recall on the high-stress class from 18% to 61%, at the cost of overall accuracy (82% → 70%; balanced accuracy 0.56 → 0.66). Deep architectures do not yet outperform tree-based methods on this sample size. These results are still being refined through further hyperparameter tuning and improved deep-learning training protocols, and will be discussed in relation to the initial non-linearity hypothesis, biological interpretability of predictive spectral regions, and feasibility for routine implementation within existing milk recording infrastructure. In conclusion, non-linear machine learning meaningfully improves MIR-based prediction of blood fructosamine and provides a more sensitive qualitative readout for hair cortisol, although quantitative cortisol prediction remains challenging across all tested methods. This refines the original non-linearity hypothesis and supports MIR spectroscopy as a realistic, low-cost basis for routine welfare monitoring.

Mid-infrared spectroscopy for large-scale monitoring of urine traits in dairy cows

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Cow urine is an informative biological matrix that can be collected using non-invasive methods when sampled as spot urine. Urinary composition is used to monitor animal health, metabolic status, and renal function. Beyond disease detection, urinary traits serve as indicators for optimizing nutritional efficiency and nitrogen use in livestock, while also acting as biomarkers for heat stress and physiological adaptation in dairy cows. Urine analyses performed through standard reference methods tend to be time-consuming, expensive, and typically limited to single traits. In this context, infrared spectroscopy may offer a cost effective and rapid tool for predicting urine composition. This study aimed to assess the feasibility of using urine mid-infrared (MIR) spectra to predict urine traits. For this purpose, spot urine samples were collected from 1,271 Holstein cows in different lactation stages and parities reared in 17 farms in the northern Italy. Urine samples were analysed using the reference methods to determine pH, specific gravity, and concentrations of total nitrogen, urea, protein, creatinine, allantoin, and uric acid. Urine samples were scanned using a MilkoScan™ 7 RM (Foss Electric A/S, Hillerød, Denmark) for MIR spectra collection. Urine spectra were matched with measured urine traits and a partial least squares analysis was performed. The model was fine-tuned using 10-fold cross-validation; spectral data points were centred and scaled. The number of latent variables was set automatically but limited at a maximum of 15 to avoid overfitting. The data set was randomly split into a calibration set (including 75% of the samples) and a validation set (including 25% of the samples), ensuring similar means and standard deviations across both the sets. The highest predictive accuracy in external validation was achieved for urea ($R^2 = 0.99$) and specific gravity ($R^2 = 0.95$), while urinary nitrogen was predicted with slightly lower accuracy ($R^2 = 0.91$). Moderate performances were observed for pH ($R^2 = 0.67$), creatinine ($R^2 = 0.65$), and allantoin ($R^2 = 0.57$), whereas uric acid, protein, and protein-to-creatinine ratio were poorly predicted. Results of the present study demonstrate that urine MIR spectroscopy accurately predicts urea concentration and specific gravity, while providing useful screenings for total nitrogen and comparing group for pH, creatinine, and allantoin. The MIR instrument used in this study is routinely applied for milk analysis, including chemical composition assessment and spectral acquisition. Although urine differs in chemical composition from milk, it is also a liquid biological matrix and can be analysed using MIR spectroscopy, provided that appropriate calibration models are developed. Since this equipment is already fully integrated into the dairy supply chain, the technology could likely be adapted to urine analysis with relative ease within the existing infrastructure. Considering that spot urine samples can be easily collected by farmers or technicians, and that the predictive models developed here provide a rapid, non-invasive, and cost-effective approach, these results highlight the potential for a scalable solution to monitor urinary traits in dairy cows. This study received support from the project Cool-Cow 2023 of the Breeders Association of Veneto Region (ARAV, Vicenza, Italy).

Prediction of Dry Matter Intake Based on Milk Mid-Infrared Spectra in Holstein Cattle

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Dry matter intake (DMI) is the key component for evaluating feed efficiency of dairy cows, yet on-farm measurement is costly and requires specialized equipment. Milk mid-infrared spectra (MIRS), routinely collected for milk testing, have been used to predict major milk components and, more recently, traits such as methane production and DMI. The objectives of this study were to develop and apply prediction equations for DMI in Canadian Holstein cows based on MIRS or milk production traits. The development dataset included 21,688 records from 1,526 first-lactation Holstein cows in three herds, with DMI, milk MIRS, milk yield, and milk composition recorded from 2018 to 2025. Daily DMI (kg/d) was averaged over seven days and analyzed for two lactation periods: 5-60 (DMI60) and 61-305 (DMI305) days in milk. Partial least squares regression (PLSR) models were built using either milk processed MIRS (512 wavenumbers) or milk production traits (milk, fat and protein yields) as predictors. Models were trained and validated using 10-fold cross-validation on 80% of the data and externally validated on 20% of the data, with the split repeated 10 times. The resulting prediction equations were then applied nationally to test-day milk records from 2018 to 2025 (5,555,183 records from 1,166,510 first-lactation Holstein cows in 6,813 herds not including the 3 herds used for model construction). In the development dataset, observed and predicted DMI had identical means (17.7 kg/d for DMI60 and 21.9 kg/d for DMI305), but predicted values showed reduced dispersion (SD 2.2 to 2.8 kg/d) relative to measured DMI (SD 4.0 to 4.2 kg/d). Using MIRS as predictors yielded external-validation correlations (r) of 0.68 ± 0.02 for DMI60 and 0.60 ± 0.01 for DMI305. Models based on milk production traits showed lower predictive performance for both traits (r = 0.53 ± 0.03 and 0.57 ± 0.02, respectively). MIRS-based predictions had an external-validation mean absolute error of 2.20 ± 0.08 kg/d for DMI60 and 2.56 ± 0.08 kg/d for DMI305, whereas milk production-based models had higher errors (2.63 ± 0.07 and 2.57 ± 0.11 kg/d, respectively). In addition, DMI predicted from MIRS and from milk production traits were moderately correlated (r = 0.51 for DMI60 and 0.63 for DMI305). At the national scale, MIRS-based predicted DMI differed by about ±1-2 kg/d from the development dataset means (18.7 ± 3.3 kg/d for DMI60; 20.3 ± 2.9 kg/d for DMI305), whereas milk production-based predictions differed less from the mean. Weak correlations (<0.20) between DMI predicted by MIRS or milk production traits at the national level indicated that these proxies were not interchangeable across all different Canadian conditions. In summary, routinely-collected MIRS supported reasonably accurate DMI prediction, particularly in early lactation, and outperformed models based solely on the major milk production traits. This approach provided DMI proxies for millions of test-day records. Further work will compare sire evaluations for predicted DMI with official breeding values based on measured DMI to assess the relevance of these proxies for genetic applications. The large-scale availability of predicted DMI also created new opportunities to investigate environmental influences on feed intake, including heat stress effects.

Decoding lipolysis through individual free fatty FT-MIR model: Enhancing predictive accuracy through real-sample enrichment and advanced algorithms

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Lipolysis is a longstanding and topic of interest within the dairy industry. Having been extensively researched throughout the twentieth century, lipolysis is often prematurely dismissed as a settled or outdated field of study. In recent years, the advent of robotic milking systems and their related increase of milking frequencies have led to a rise in lipolysis levels, sparking renewed interest in monitoring milk quality. For individual cows, this monitoring could help to select cows who could resist to lipolysis and/or highlight bad farming practices. Predicting lipolysis more effectively relies on the analysis of free fatty acid (FFA) concentrations. Recent research has demonstrated the feasibility of quantifying individual free fatty acids in milk; specifically, the use of Fourier Transform mid-infrared (FT-MIR) spectroscopy on milk has shown promising results in predicting these traits. But this research has several limitations such as the use of unofficial reference analysis and the use of artificial lipolysis samples to gain in variability. To overcome these limitations, individual FFA models developed previously have been used to select, through the Walloon milk recording, 165 samples with a predicted high lipolysis level. Moreover, 41 samples were collected at the local farm of the CRA-W. All samples have been analyzed using the official reference analysis. FFA FT-MIR models built using Partial Least squares (PLS) regression and only the new dataset give the same performance than the literature. To improve the prediction performances as non-linear relationships can exist, advanced machine learning algorithms were tested using the full dataset (N=1004). PLS regression, Kernel Ridge Regression (KRR), Gaussian process regression (GPR) and Support vector machine Regression (SVR) give better performance than other literature models with R^2CV of 0.72, 0.5 and 0.45 for Short Chain FFA, Mid Chain FFA and Long chain FFA respectively. Adding real lipolysis samples lead to a more robust models without changing the performances. This refinement of lipolysis has resulted in improved oversight of agricultural practices and enhanced valorization of milk for the purpose of its transformation.

Using Fourier Transform Infrared (FTIR) Spectra to Investigate GXE Interactions in Danish Holstein Cattle

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Genotype-environment interaction (G×E) plays a significant role in milk production systems because animals can respond differently to changing climatic conditions. In dairy cattle, it is often observed that current practices overlook this aspect, assuming that the same genotype will be effective across all environments. However, G×E interactions can often be a significant source of variability in productivity and reproductive performance. Understanding G×E interactions is important, especially for changing dairy cattle breeding practices. In recent years, Fourier-transform infrared (FTIR) spectra have begun to provide a new perspective not only for routine analysis of milk composition but also for the analysis of complex phenotypes with low predictability, such as disease incidence, by integrating genomic data with high-throughput phenomic information. This can serve as an indirect indicator to characterize the environmental variation that animals are exposed to. The aim of this study is to evaluate the integration of FTIR spectral data into genomic prediction models and to examine its impact on prediction accuracy across herds, considering potential genotype-environment interactions. Mastitis was used as a model trait to evaluate this approach. The dataset includes test day records (39 000) for milk yield (MY), clinical mastitis cases, and somatic cell count of 10 000 Holstein animals as well as infrared spectral data in the period of October 2023 to December 2024. Spectral data were obtained using MilkoScan FT2 (FOSS Analytical A/S), which captures infrared absorbance in the range of 5008 to 925 cm□¹, encompassing a total of 1060 individual wavelengths. Genotypes (50K SNP chip) and pedigree data were available for all animals. In this study, environmental information was extracted from phenotypic and spectral data. This study employed a kernel-based genomic prediction approach integrating milk FTIR spectral data. Preliminary findings for SCC showed a heritability of 0.08 (SE 0.02) when including a GRM only in the model. The variance explained by the IRM for SCC was 0.63, indicating that the infra-red spectra can explain some of the variance of SCC. Including both the GRM and IRM in the model showed that the GRM took most of the variance while the IRM variance went to zero. Further analyses are ongoing to evaluate model performance and investigate the possibility to use milk infrared spectra data in relation to the prediction of genotype-environment interaction.

Comparing spectral acquisition methods using a handheld NIR spectrometer for estimating legume content from legume–grass mixtures in Northern Sweden

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A proper legume content in pastures is essential for meeting livestock nutritional requirements and promoting sustainable pasture. The legume proportion in legume-grass mixtures can be rapidly assessed using near-infrared (NIR) spectroscopy. However, NIR predictive performance is highly sensitive to the spectral acquisition method. Previous studies have investigated the use of handheld NIR spectrometers, focusing on various scanning techniques for forage materials in dried samples. On the other hand, literature on using hand-held NIR spectrometer for estimating botanical composition from fresh pasture samples is limited, with few studies directly comparing different spectral acquisition techniques. The aim of this study was to compare two spectral acquisition methods – stationary (ST) and sliding (SL) – using a handheld NIR spectrometer for estimating legume content in fresh legume-grass mixtures in Northern Sweden. A total of 112 forage samples was collected from 12 pasture fields located in Umeå, Västerbotten County (Sweden). Fields were sown with a seed mixture of a 40% timothy (Phleum pratense L.), 40% meadow fescue (Festuca pratensis), 10% Kentucky bluegrass (Poa pratensis) and 10% white clover (Trifolium repens). Sampling was performed on three occasions from six random points on each field, using a 50 x 50 cm quadrat. The plant material within each quadrat was cut at 3.0 cm height, and the samples were manually sorted to exclude weeds and dead material. Spectral data were collected with a NeoSpectra spectrometer in the 1350-2500 nm range, using both ST and SL scanning methods. For the ST spectra collection method, the spectrometer was placed at four randomly selected points on the sample container, while for the SL technique, the spectrometer was moved over the sample surface while maintaining close contact with the forage. After scanning, each sample was manually sorted into grass and legume, and spectra from synthetic samples with 100% clover were collected (n=26). Samples were then oven-dried at 105°C for 48 hour and weighed to determine the percentage for each botanical category. The averaged spectra for each sample (138 in total), from both scanning techniques, were used to develop predictive models for legume proportion determination using partial least squares (PLS) regression. Model predictive ability was evaluated using the coefficient of determination (R^2), root mean square error (RMSE), and residual prediction deviation (RPD). The SL technique showed decreasing absorbance with increasing clover proportion, whereas ST spectra were more similar across grass proportions, suggesting improved discrimination with SL. This was confirmed by the PLS results: the SL achieved higher predictive accuracy (R^2=0.84, RMSE=11.07, and RPD=2.83) than the ST method (R^2=0.79, RMSE=17.02, and RPD=2.19). In conclusion, although both acquisition methods provided satisfactory predictions of legume proportion, the sliding technique showed a superior discriminative ability and better predictive performance in estimating legume proportion in fresh pasture samples.

BROMEDIR: Development and evaluation of a miniaturized FT-MIR instrument for in-farm milk analysis

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Mid Infrared (MIR) analysis of milk is a powerful technology to predict a wide range of phenotypes related to fine milk composition, cow health, nutrition and environmental footprint. MIR analysis of individual cow milk samples is used by many milk recording organizations around the world for cow management. However, milk recording is classically organized every 4 to 6 weeks in farms, while to enhance the capacity of MIR analysis to be used for precision livestock farming (e.g. for early detection of diseases), the frequency of analysis should be increased. A potential solution to achieve such high frequency measurements is to bring the MIR spectrometer directly to the farm to enable daily analyses. The BROMEDIR project aims to fill this gap by developing a portable miniaturized spectrometer operating in a very wide spectral range (5000-1000 cm^{-1}), dedicated among other applications to milk analysis. Thanks to the recent technological miniaturization advances, a prototype was developed to meet dairy stakeholders' requirements and affordable cost. The objective of this work is to develop and assess the performance of the miniaturized portable MIR spectrometer in milk application. The instrument is evaluated, among other criteria, based on the general spectral pattern, the practical signal to noise ratio, the repeatability and reproducibility over time of both the spectral response and the main milk components. First results reveal a spectral profile comparable to that obtained with benchtop instruments, with identical peak assignments. Although the signal-to-noise ratio in the fingerprint region is lower than that of benchtop systems, the prototype exhibits a higher signal-to-noise ratio in the lipid-associated peak regions. The accuracy of the spectrometer to predict the main milk components is evaluated against benchtop predictions after the analysis of 50 raw milk samples from individual cows. The linearity of the peaks at 2927 and 1550 cm^{-1} with fat and protein contents, respectively, is demonstrated by coefficients of determination exceeding 0.9. Finally, the possibility to transfer existing MIR models is assessed through standardization of the BROMEDIR spectrometer against benchtop instruments. This approach would open great potential by enabling the use of past developments within the FT-MIR milk analysis framework (e.g. milk fatty acid profile and health of cows, among other).

Towards a single-step genomic evaluation of cheese-making traits predicted from MIR spectra in eight French dairy cattle breeds

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The FROM'MIR project (2015–2019) developed equations predicting cheese-making properties (CMP) from mid-infrared (MIR) spectra in the Montbéliarde breed, enabling the first genomic evaluation for these traits. The APIS-GENE–funded FROM4ALL project extends these predictive models and genomic tools to eight additional French dairy breeds (Abondance, Jersey, Brown Swiss, Tarentaise, Simmental, Normande, Vosgienne, and Holstein). Six CMP were predicted from MIR spectra: fresh curd yield (CYfresh), curd yield in dry matter (CYDM), ratio of curd firming time to coagulation time for soft cheese (K10/RTCSC), curd firmness at RCT for soft cheese (aSC), curd firmness at RCT for pressed cooked cheese (aPCC), and curd firmness at 2×RCT for soft cheese (a2SC). The objectives of this study were to estimate genetic parameters for these traits and to implement single-step genomic evaluations. After quality control, the dataset comprised more than 50 million test-day records across the eight breeds. For genetic parameter estimation, 15,548,851 records from 1,950,417 primiparous cows in 27,485 herds were retained, with a minimum of three test-day records per cow. Genetic parameters were estimated using a repeatability animal model implemented in Wombat. Heritability estimates were low to moderate and consistent across breeds, aligning with previous results in Montbéliarde. Estimates ranged from 0.14 to 0.63 for cheese yields and from 0.28 to 0.49 for coagulation traits. Genetic correlations displayed clear and biologically coherent patterns: coagulation traits were strongly positively correlated (0.90–1.00), except for K10/RTCSC. As expected, faster coagulation (lower K10/RTCSC) was associated with firmer curd (aSC, a2SC, aPCC). K10/RTCSC was negatively correlated with all traits (−0.62 to −0.86) indicating that faster coagulation improves curd firmness and fresh/dry matter yields. Correlations between CMP and milk production or udder health traits ranged from low to high. CMP exhibited moderate negative correlations with milk yield but generally strong positive correlations with milk protein and fat contents. Associations with somatic cell count were weak and occasionally inconsistent across breeds. Leveraging pedigree, phenotypic, and genotypic data, we implemented a single-step genomic evaluation using a test-day model considering the first three lactations. Depending on the breed, single-step estimated breeding values (ssEBVs) were calculated using 15,340 to 18,512,229 observations from 2,234 to 2,568,184 cows, including 1,684 to 321,895 cows with genotypes. Genetic trends of CMP traits were assessed by calculating the mean ssEBVs of bulls and cows by year of birth. They generally showed improvement or stabilization across breeds and sexes, consistently with the breeding goals aiming at increasing milk protein and fat contents. Overall, our results demonstrate that all CMP are heritable and provide a prototype of single-step genomic evaluation that can be directly implemented in routine in eight dairy cattle breeds.

From routine reference data to scalable phenotypes: multivariate models combining milk MIR spectra and cow information for metabolic monitoring

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Milk mid-infrared (MIR) spectrometry is routinely used in milk recording and enables high-throughput phenotyping. However, often prediction models for novel traits are based on limited experimental datasets and lack validation under field conditions. In the MeMoMIR project, two independently collected routine data streams—milk performance data from Qnetics, including cow information, performance traits and MIR spectra, and large-scale metabolic monitoring data from the Thuringian Animal Disease Fund—were linked to establish prediction models under practical conditions. The dataset (2020–2025) comprised 159 herds and ~17,000 cows, of which records from 119 herds and ~4,200 lactating Holstein cows (≤150 DIM) with close temporal alignment between milk and reference sampling were retained. Multivariate prediction models were developed for 23 metabolic traits using partial least squares regression, including cow-level covariates and MIR spectra. The models were extensively tuned via grid search across spectral pre-processing and model configurations. Model performance was evaluated using a strict temporal split (train: 2020–2024; test: 2025) combined with a herd-disjoint validation design to mimic prospective application under field conditions and to ensure robust assessment across heterogeneous herd environments. Predictive ability varied widely across metabolites, with R^2val ranging from 0.00 to 0.70 and a clear contrast between trait groups. The highest performance was achieved for blood cholesterol (R^2val = 0.70), followed by bilirubin (0.41), total protein (0.27) and albumin (0.26), while urea (0.23) and β-hydroxybutyrate (0.23) showed moderate predictive ability. In total, six traits reached R^2val ≥ 0.20, whereas the majority of traits showed limited predictive performance (R^2val < 0.20), indicating marked variability in predictability across metabolites and biological functions. Prediction of urine metabolites was consistently weaker than for blood-based traits, with only creatinine (0.19) and sodium (0.13) reaching R^2val values above 0.10, while several parameters such as urinary calcium (0.00) and chloride (0.00) as well as blood creatine kinase (0.00) showed negligible predictive ability. Notably, some traits (e.g. bilirubin) showed comparable or slightly higher performance in external validation than in cross-validation, indicating robust generalization under the applied validation scheme despite heterogeneous and non-experimental field data. Predictive performance may be constrained by limited biological variability, as routine monitoring predominantly includes clinically unaffected animals and thus underrepresents extreme metabolic states. These results demonstrate that MIR-based prediction under real-world conditions is feasible for selected metabolic traits in blood, while performance remains strongly trait-dependent and limited for many other analytes. The integration of previously independent routine data streams provides a unique and scalable framework for continuous model evaluation and improvement under field conditions, thereby defining both the potential and current boundaries of metabolic phenotyping from routine data. Further model refinement and targeted inclusion of animals with more diverse metabolic states are ongoing.

The efficacy of kenaf leaf meal on the apparent nutrient digestibility and physiological parameters of New Zealand white rabbit bucks

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Abstract The current study aimed to evaluate the effects of varying levels of kenaf leaf meal (KLM) on apparent nutrient digestibility, growth performance, and haem-biochemical indices in New Zealand White Rabbit bucks (NZWRB). The authors hypothesised that varying levels of KLM have no significant effect on apparent nutrient digestibility, growth performance and haem-biochemical indices of NZWRB. Mature kenaf leaves were harvested, air-dried, and milled to produce KLM. The KLM was chemically characterised and further used to produce four KLM-based experimental diets by partially replacing soyabean meal (SMB) in rabbit grower diet: KLM0% – rabbit grower diet without KLM as substitute, KLM2.5% – rabbits grower diet with 2.5% KLM as substitute, KLM5.0% – rabbit grower diet with 5.0% as a substitute and KLM7.5% – rabbit grower diet with 7.5% as a substitute. Twenty-eight (28) NZWRB at fourteen weeks of age with an initial weight of 2.3 ± 0.47 kg, were randomly allocated to four experimental diets (KLM0%, KLM2.5%, KLM5.0% and KLM7.5%) in a complete randomised design (CRD) for a period of 70 days. The experimental unit was a pen (0.07 m2 per buck) housing one NZWRB buck, replicated seven (7) times. The collected data on apparent nutrient digestibility, growth performance, and haem-biochemical parameters were statistically analysed using SAS, version 9.2.1 (2010). The means were separated using Duncan's multiple range test, and significance was considered at $p < 0.05$. The results showed that rabbits fed KLM5.0 and KLM7.5% had the highest ($p < 0.05$) crude protein, lysine, aspartic acid, glutamine and copper digestibility, while those fed KLM2.5% had the highest ($p < 0.05$) leucine digestibility. Rabbits fed KLM7.5% had the highest ($p < 0.05$) weight gain at week 6, while those fed KLM2.5, KLM5.0, and KLM7.5% had the highest weight gain at week 10. Haemoglobin was highest in rabbits fed KLM2.5 and KLM5%, while red blood cell count was highest ($p < 0.05$) in rabbits fed KLM5.0%. Rabbits fed KLM2.5 and KLM5.0% highest white blood cell count, while those fed KLM7.5% had the highest ($p < 0.05$) blood phenolics and antioxidants. Blood potassium and aspartate transferase were highest ($p < 0.05$) in rabbits fed KLM2.5 and KLM5.0%, while rabbit bucks fed KLM5.0% had the highest ($p < 0.05$) creatine kinase. Rabbits fed KLM5.0 and KLM7.5% had the highest ($p < 0.05$) blood glucose and iron. The KLM5.0 and KLM7.5% showed improved nutrient digestibility and growth performance, indicating that KLM can be a valuable feed ingredient for rabbits. Therefore, it can be gradually incorporated into the rabbit diet up to 7.5% without negatively impacting the overall growth and health performance. Keywords: Growth performance, kenaf leaf meal, soyabean meal, nutrient digestibility, haem-biochemical indices, health performance.

Performance of rabbit does fed a lactation diet based primarily on agro-food by-products: implications for a circular economy

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Within the framework of a circular bioeconomy, population growth and increasing resource constraints are expected to shift livestock production, including rabbit meat production, toward circular models that prioritise locally available protein sources and agro-industrial by-products. Rabbit nutrition may serve as a suitable model for this approach. This study compared a conventional lactation diet (CL diet; alfalfa meal, soybean meal, wheat bran, sugar beet pulp, oats, barley, and rapeseed oil) with a lactation diet based primarily on agro-food by-products (L-AFB diet; rapeseed cake, wheat bran, sugar beet pulp, maize bran and flour, buckwheat hulls, and sunflower hulls), with alfalfa meal, soybean meal, oats, barley, rapeseed oil, and supplemental dicalcium phosphate completely excluded. The diets were comparable in crude protein, fibre fractions, limiting amino acids, calcium, and phosphorus. The L-AFB diet contained slightly more ether extract and less starch and digestible protein than the CL diet. Both diets were isoenergetic. A total of 32 rabbit does (16 per treatment; 4th parturition) were fed one of the two diets throughout the 32-day lactation period. Does were housed in modified cages allowing controlled suckling and separate access to feed for does and their litters. Litters were standardised to 10 kits at birth and received their mothers' lactation diets from day 17. Milk composition was analysed in five does per treatment. Dietary treatments did not affect doe live weight at the end of lactation (on average 4795 g), average daily feed intake (on average 434 g), average daily milk production (on average 274 g), or milk, fat, and protein output (on average 77.7, 9.3, and 7.3 g/d/kg metabolic weight, respectively). Litter live weight at 32 days (weaning) was also unaffected by the dietary treatments (on average 6945 g). Between days 22 and 32 of lactation, litters from does fed the L-AFB diet consumed slightly less solid feed (by 2.8 g/d; $P = 0.020$) and had a higher milk-to-solid feed intake ratio (by 0.26; $P = 0.016$). These results support the idea that diets based primarily on agro-food by-products can represent a viable alternative feeding strategy for rabbit does, providing an environmentally friendly approach to rabbit meat production. The study was supported by a project NAZV QL 24010136.

Dietary evaluation of Pleurotus ostreatus spent mushroom substrate as a sustainable feed ingredient for enhancing performance, egg oxidative stability and ovarian homeostasis in laying hens

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The valorization of agro-industrial by-products in poultry nutrition aligns with circular bioeconomy principles and sustainable production strategies. This study evaluated the dietary inclusion of Pleurotus ostreatus spent mushroom substrate (SMS) on laying performance, nutrient digestibility and egg quality. Additionally, key ovarian homeostasis-related genes involved in detoxification (aryl hydrocarbon receptor-AhR), antioxidant (nuclear factor erythroid 2–related factor 2-Nrf2), and inflammatory response (nuclear factor kappa B-NF-κB) were assessed. A total of 196 Hy-Line Brown laying hens (51 weeks of age) were allocated to 4 dietary treatments with 7 replicates of 7 hens each, receiving isocaloric, isonitrogenous and isofibrous diets containing 0 (S0), 25 (S25), 50 (S50), or 100 (S100) g SMS/kg diet for 7 weeks. Productive performance was recorded daily and was reported for the overall period, while total tract apparent nutrient digestibility was determined at week 7. At the end of the trial (week 7), eggs were collected to assess quality and oxidative stability in fresh and stored conditions, and ovarian samples were obtained for qPCR analysis. Data were analyzed using ANOVA with means compared by Tukey's honest significant difference test. Polynomial contrasts tested the linear and quadratic effects of SMS inclusion levels. Increasing SMS inclusion improved laying rate and egg mass ($P \leq 0.05$), with the highest responses observed in S100 compared with the control treatment (S0). Ether extract digestibility was enhanced ($P = 0.002$) in all SMS-supplemented treatments compared with S0. Egg oxidative stability was improved ($P \leq 0.001$), as reflected by reduced yolk malondialdehyde and egg-white protein carbonyl concentrations in fresh and stored eggs, particularly in S100. The nutrigenomic analyses revealed that SMS inclusion downregulated most of the AhR (3/4) and NF-κB (8/13) pathway genes assessed, mainly in S50 and S100 treatments. In addition, SMS-related cytoprotective potential was demonstrated via beneficial changes seen for the majority (5/9) of the Nrf2 pathway genes assessed, with S100 displaying the most significant differences from S0. In conclusion, dietary SMS inclusion at 100 g/kg improved layer productivity, ether extract digestibility and egg oxidative stability. These responses were mechanistically supported by beneficial modulation of ovarian cytoprotective and inflammatory responses. Overall, these findings support the use of SMS as a sustainable functional feed ingredient to enhance poultry productivity, egg quality and physiological resilience.

Impact of Moringa oleifera leaf meal supplementation on the growth, nutrient digestibility, and meat quality of quails fed sorghum-canola-based diets

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Abstract Sorghum and canola meal are low in xanthophyll and carotenes which are responsible for egg pigmentation. It is, therefore, imperative that alternative and sustainable way of incorporating a safer and local phytogenic plant should be implemented to avoid white yolk which could lead to eggs being rejected by the consumer in the market. No research has been conducted world-wide on the feeding quail's sorghum-canola based diets supplemented with Moringa oleifera leaves meal (MOLM) in South Africa. The aim of the study was to investigate the effects of feeding Japanese quail's sorghum-canola based diets supplemented with MOLM as a feed additive on nutrient digestibility, growth performance, egg production, carcass characteristics and meat quality. A total of 140 Japanese quails (42.7 ± 4.81g of live weight) were reared on 5 iso-caloric and iso-nitrogenous diets formulated by using maize + soybean and white sorghum + canola meal supplemented with MOLM. The study was separated into two experiments: 1A – quails raised on diets without MOLM supplementation from week 4-8 and 1B quails raised on diets with MOLM supplementation from week 9-12. Growth performance and nutrient digestibility and egg production were measured for 5 weeks. Thereafter, the quails were humane slaughtered for carcass traits and meat quality. The collected data were statistically analysed using SAS, version 9.2.1. The means were separated using Duncans's multiple range test, and significance was considered at $p < 0.05$. In experiment 1A, protein efficiency ratio (PER) was the highest on quails reared on sorghum- canola based diets (SCD) in week 8. In experiment 1B, with MOML supplementation, crude protein digestibility was the highest in quails reared maize-soyabean based diets supplemented with 25% (MBD25), while overall weight gain and egg production was the highest on quails reared on maize-soybean based diets supplemented with 50% (MBD50) MOLM. Protein consumed was lower ($p < 0.05$) on quail reared on maize-soybean based diets supplemented with 50% MOLM while PER was the highest on sorghum-canola based diets (SCD) supplemented with 50% MOLM. Quails fed CM50 had the shorter ($p < 0.05$) small intestine compared to those fed other treatment diets. Meat b* was significantly lowest from quails fed maize soybean-based diets supplemented with 25% MOLM. Meat pH24 was lowest ($p<0.05$) from quail fed MBD50. Based on nutrient digestibility, weight gain and egg production, it can be concluded that maize soybean-based diet supplemented with 50% MOLM does not result in adverse effects on diet utilisation, growth performance and meat quality of quails. Anti- nutritional factors in sorghum canola-based diets need to be ameliorated before being used as energy sources. Keywords: Quails, nutrient digestibility, growth performance, egg, meat, canola meal, Moringa oleifera leave meal

Environmental Sustainability and Functional Performance of Alternative Feed Ingredients: An Evidence Synthesis

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Background Alternative feed ingredients such as macroalgae, algal oils, single-cell proteins, and insects are increasingly proposed as sustainable alternatives to conventional feed ingredients. However, environmental impacts vary widely depending on production systems and feedstocks used, energy sources, and processing pathways. Circular production approaches, such as using by-products, residual streams, and wastes as inputs and valorising co-products, have therefore emerged as strategies to improve the sustainability of feed ingredient production. At the same time, questions remain regarding the nutritional adequacy and performance of feeds derived from these alternative ingredients. While nutritional profiles can vary depending on production processes and may be optimised through formulation, evidence linking environmental and animal nutrition outcomes remains fragmented across disciplines. In addition, methodological differences across life-cycle assessments (LCAs) and feed trials limit comparability between ingredients and their underlying production systems. The aim of this study was to compare emerging alternative feed ingredients used in livestock, aquaculture, and pet food systems in terms of their ability to support both sustainability and animal health outcomes. The objectives of this study were to: (1) evaluate the environmental sustainability of selected alternative feed ingredients across key impact domains; (2) examine how variation in production conditions, including circular production pathways, influences sustainability performance; and (3) assess whether lower-impact ingredients and production pathways are compatible with acceptable animal health and performance outcomes. Methods We conducted a structured synthesis of peer-reviewed LCAs and sustainability studies reporting greenhouse gas emissions, land use, energy use, and water use. Environmental indicators were standardized to comparable functional units and analysed across production system characteristics, including feedstock, energy source, and processing pathway. In parallel, we reviewed peer-reviewed feeding trials assessing outcomes including digestibility, palatability, gastrointestinal tolerance, and growth performance. Results Environmental outcomes varied widely across ingredients and were strongly influenced by production systems. Key drivers of environmental impacts included energy sources, feedstock, processing intensity, and co-product management. Production systems integrating renewable energy or valorising by-products generally showed more favourable environmental profiles. Evidence from nutrition studies suggests that low to moderate inclusion of alternative feed ingredients is generally well tolerated across a number of species and can, in some cases, support improved growth or health outcomes. At higher inclusion levels, results are more variable, with the common limitations relating to growth rates, palatability and gastrointestinal tolerance. Discussion/Conclusion Overall, the findings suggest that alternative feed ingredients can support both sustainability and animal health goals, but outcomes depend strongly on how ingredients are produced and used. More circular and lower-impact production pathways appear promising, particularly when paired with low to moderate inclusion levels that maintain acceptable nutritional performance. This highlights the need to assess alternative ingredients not as uniform categories, but in relation to the specific production systems and feeding strategies under which they are deployed.

Alternative legumes as a sustainable replacement for soybean meal in Iberian pig diets: effects on growth performance and plasma metabolites

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Legume grains can be an excellent source of protein in animal diets. The scientific community is adopting strategies for the cultivation, production and use of local legumes as potential feed protein sources aiming to improve the sustainability of feed and livestock production chains, affected by production costs, market instability and long-distance transportation of feedstuffs such as cereals and soybeans, that are mainly produced in North and South America. Our aim was to evaluate local protein sources, such as peas, chickpeas and lentils, as a sustainable alternative to soya through the impact on growth performance and plasma metabolites of Iberian pigs, a relevant Spanish native pig breed. A total of thirty-two pure Iberian barrows were randomly allocated into four experimental groups: a control group fed soybean meal and three groups in which 50% of soybean protein was replaced by local legume protein (peas, cull chickpeas and cull lentils). Diets were isoproteic and isocaloric, covering animals' needs and formulated according to the ideal protein concept. Pigs were individually housed under temperature-controlled conditions (22 ± 1ºC) with access to feed and water ad libitum and weighed weekly. Initial weight was 33 ± 1.3 kg and final weight was 74 ± 2.5 kg. 10 weeks after the beginning of the trial, animals were slaughtered after 24 h fasting by electrical stunning and immediate exsanguination. Blood samples were processed to analyze biochemical parameters: albumin, β-hydroxybutyrate, HDL-cholesterol, LDL-cholesterol, total cholesterol, creatine, glucose, lactate, triglycerides and urea. Treatment effects were assessed by analysis of variance with a significance level of 0.05. No significant differences were found among treatments for average weight gain (41.4, 40.8, 40.8 and 42.5 kg, for control, chickpea, pea and lentil groups, respectively; standard error of the mean (SEM): 1.49; $P > 0.05$), average daily gain (820, 810, 810, 842 g/day, for control, chickpea, pea and lentil groups, respectively; SEM: 34.9; $P > 0.10$), average feed intake (2775, 2716, 2783, 2848 g of dry matter/day, for control, chickpea, pea and lentil groups, respectively; SEM: 100.5; $P > 0.10$) and feed efficiency (g/g) (0.296, 0.291, 0.297, 0.295, for control, chickpea, pea and lentil groups, respectively; SEM: 0.006; $P > 0.10$). Likewise, there were no differences in the plasma biochemical metabolites ($P > 0.10$), except for creatinine, which was lower in the control and lentil group, compared to chickpea and pea groups (0.60, 0.62, 0.73, 0.74, respectively; SEM: 0.04; $P < 0.05$), and a numerical trend suggesting that lentils reduced plasma triglycerides ($P = 0.065$). The present results indicate that substitution of 50% of soy protein with locally grown protein did not negatively affect growth performance or plasma biochemical parameters. These finding suggests that incorporating local legumes into pig diets could enhance the sustainability of the pig production chain without compromising animal performance. Funded by the EU project Facilitating Innovations for Resilient Livestock Farming Systems, abbreviation: Re-Livestock (reference number: 101059609).

Alternative legumes as a sustainable replacement for soybean meal in Iberian pig diets: effects on nutrient digestibility, nitrogen balance and inflammatory markers

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Legume grains can be an excellent source of protein in animal diets. The scientific community is adopting strategies for the cultivation, production and use of local legumes as potential feed protein sources aiming to improve the sustainability of feed and livestock production chains, affected by production costs, market instability and long-distance transportation of feedstuffs such as cereals and soybean, that are mainly produced in North and South America. Our objective was to assess local protein sources –such as peas, chickpeas, and lentils- as sustainable alternatives to soybean by evaluating their effects on nutrient digestibility, nitrogen balance, and intestinal inflammation markers in Iberian pigs, an important native Spanish breed. A total of thirty-two pure Iberian barrows were randomly allocated into four experimental groups: a control group fed diets with soybean meal as the main protein source and three groups in which 50% of soybean protein was replaced by local legume protein (peas, culled chickpeas and culled lentils). Diets were isoproteic and isocaloric, covering animals' needs and formulated according to the ideal protein concept. Pigs were individually housed under temperature-controlled conditions (22 ± 1ºC) with access to feed and water ad libitum. Initial weight was 33 ± 1.3 kg and final weight was 74 ± 2.5 kg. Three weeks after the beginning of the trial, faecal digestibility and nitrogen balance were assessed in 24 animals (6 per treatment). Samples of faeces and urine were obtained quantitatively for 4 consecutive days. Excreta samples were homogenized and freeze-dried to determine dry matter, organic matter, nitrogen and energy ingested and excreted in faeces and nitrogen and energy excreted in urine. At the end of the 10-week trial, pigs were slaughtered and samples of faeces from the rectum collected for biochemical analysis of two markers of intestinal inflammation: calprotectin and myeloperoxidase. Treatment effects were assessed by analysis of variance with a significance level of 0.05. No significant differences were found between the total tract apparent digestibility coefficients for dry matter (mean value: 0.862; standard error of the mean (SEM): 0.009; $P > 0.10$), organic matter (mean value: 0.883; SEM: 0.008; $P > 0.10$), nitrogen (mean value: 0.801; SEM: 0.015; $P > 0.10$) or energy (mean value: 0.861; SEM: 0.009; $P > 0.10$). Furthermore, no differences were observed on the amount of ingested nitrogen nor retained nitrogen ($P > 0.05$). Likewise, the values for retained nitrogen/ingested nitrogen (mean value: 0.451; SEM: 0.042) and retained nitrogen/digested nitrogen (0.562; SEM: 0.049) were not significantly different between groups ($P > 0.10$). Finally, faecal calprotectin concentrations were significantly higher in the control group compared with the local-legume fed groups ($P < 0.10$). However, myeloperoxidase levels remained unaffected ($P > 0.10$). These results suggest that replacement of 50% of soy protein with protein from locally-grown legumes did not impair nutrient digestibility nor nitrogen balance and may improve gut inflammation enhancing the sustainability of pig production. Funded by the EU project Facilitating Innovations for Resilient Livestock Farming Systems, abbreviation: Re-Livestock (reference number: 101059609).

From circularity to practical application: optimizing food industry co-products for pig and poultry feeding

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The use of agro-industrial co-products and other food-derived ingredients in feeds for pigs and poultry has been widely recognized as a feeding strategy that contributes to circularity and reduces the environmental impact of feed production. The main reason is that these ingredients originate from other industrial processes and require fewer resources -such as land, water, or fuel- than conventional soybean meal and cereals for their production. Additionally, they are generally not suitable for human consumption and are available in local markets, thereby reducing food-feed competition and reliance on imported raw materials. These issues are particularly significant in the current demographic and geopolitical situation. For all these reasons, the incorporation of by-products derived from crop cultivation, as well as from fruit, vegetables, and other food-processing industries, into animal feeding has become a central topic in both European and global discussions. The research conducted in our laboratory for more than 15 years on agro-industrial co-products such as citrus pulp, olive cake, brewery spent grains, rapeseed and camelina meals, among others, has shown that, beyond being nutrient-rich resources, they can provide several additional advantages related to animal health, meat or egg quality, and gas emissions from slurry. Owing to their composition, which is typically rich in fibre fractions and bioactive constituents such as phenolic compounds, their inclusion in diets can positively modulate and support a favourable gut microbiota composition and functionality, activating metabolic pathways beneficial for pigs' health. In addition, co-products in feeds have the capacity to mitigate ammonia emissions from slurry, although their effects on methane emissions are more variable. However, these alternative ingredients are also widely known to present certain limitations, including issues related to availability, variability, the need for appropriate preprocessing in most cases, and constraints in nutrient availability. This latter aspect is particularly relevant for monogastric animals, as it can negatively affect nutrient digestibility and increase the overall amount of excreta, potentially reducing the environmental benefits typically attributed to the use of by-products. Factors such as the amount and type of fibre, as well as their physicochemical characteristics can contribute to this issue, and the inclusion level in the diet plays a major role in it. Addressing these issues is essential for ensuring the effective and sustainable integration of co-products into livestock diets. Extensive research is currently being conducted in the field of emerging technologies and innovative approaches to optimize their nutritional value. These innovations include treatments such as fermentation or micronutrient biofortification, as well as the use of exogenous enzymes, such as specific fibrolytic enzymes, capable of degrading both soluble and insoluble fibre. Additionally, artificial intelligence tools, including machine learning algorithms, have recently been applied to predict nutrient deficiencies and optimize diet formulation based on animal responses. With sufficient data, these models can identify optimal feedstuff combinations to maximize efficiency, helping determine suitable feeding strategies that incorporate by-products and assess their broader sustainability impacts. Future research should focus on better characterizing agro-industrial and other food industry co-products and its effects, and refining feeding strategies with these ingredients using available technological and digital tools to enable their practical adoption on farms.

Hybrid rye inclusion in fattening pig diets to reduce environmental impacts

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Diversification of rotations is a main lever to improve sustainability of agriculture, through improved soil health, reduced used of pesticides and fertilizers, and improved yields. Fall hybrid rye is a good candidate crop to introduce in conventional rotations, with low input requirements, improved soil coverage and higher yields compared to other winter cereals. However, a main barrier to adoption is lack of market opportunities to sell these crops. Animal feed, and more precisely pig feed, could be a good market as pigs fed hybrid rye have shown good performance results, and replacing corn with rye could reduce the environmental impact of feed and pig production overall. The objectives of this study were to quantify the environmental impacts of introducing hybrid rye in a Corn-Soybean rotation in Quebec (Canada) and to evaluate the environmental impact reductions associated with replacing corn with local rye in pig feed. Life cycle assessments (LCAs) based on primary data were carried out using SimaPro® software, comparing a corn-soybean rotation and a corn-soybean-hybrid rye + legume cover crop. Impacts of hybrid rye produced in Quebec was calculated based on a harvest-to-harvest year. Impacts taking into account benefits of the rotation were calculated by allocating reduction of corn and soybean impacts to rye. Iso-net-energy and iso-digestible-lysine fattening pig diets were formulated with 25% or 50% of corn replaced by rye (S25 and S50 respectively), compared to a control that represented local practices (four feeding phases). A farm-gate LCA of these strategies was performed assuming no effect on performance and using rye LCA values calculated in this study and Tier 3 modelling of manure composition and emissions. Impacts were expressed per kg of bodyweight. The ReCiPe characterization method was used. One kg of rye had lower impacts than 1 kg of corn, except for land occupation. Climate change impact was 19% lower, freshwater ecotoxicity 13% lower and eutrophication 3% lower. Including rotation benefits decreased rye impacts by more than 30% for all impacts except ecotoxicity. When considering harvest-to-harvest rye impact values, most impacts of pig production were reduced: climate change impact was reduced by 3% for S25 and 6% for S50, eutrophication was reduced by 6% and 16% for S25 and S50, respectively, fine particle formation by 5% and 8%, ecotoxicity by 6% and 11%, fossil resource depletion by 7% and 17%, acidification by 4% for S25 and 5% for S50. Land use increased by 3% and 6% for the two replacement levels, respectively. When allocating rotation benefits, impact reductions were higher, especially for eutrophication: 36% of reduction for S25 and 77% for S50. Fossil resource depletion was reduced by 12% and 27% for S25 and S50, respectively, and climate change by 6% and 12%. Land use was reduced for S25 and S50 with these impact values of rye. Other impacts were reduced by between 6% and 8% for S25 and between 10% and 15% with S50. Adding hybrid rye to crop rotations and pig diets in Quebec is an efficient strategy to decrease environmental impacts of the sector, with no major trade-offs between impacts or to animal performance.

Meta-Analytical Prediction of Metabolizable Energy in Solvent-Extracted Canola Meal pigs Using Chemical Composition

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The shifting from fossil-derived sources to plant-based alternatives has increased the production of oilseed and, consequently, the availability of oilseed meals. In Canada, canola meal (CM) is a by-product of canola seed oil extraction and an important protein source for livestock, including swine. Optimizing the nutritional use of CM is critical for enhancing its value for canola production chain and the swine industry. However, variability in its chemical composition can influence its metabolizable energy (ME) contribution to swine diets. Therefore, the objective of this study was to predict the ME of CM for swine based on its nutrient composition using a meta-analysis approach. A dataset comprising 19 studies (56 experimental treatments) that reported ME values of solvent-extracted CM for pigs between 20 and 50 kg body weight, along with chemical composition data (crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF), ether extract (EE), and ash), was compiled. This dataset was extracted from an initial collection of publications addressing nutrient and energy digestibility of canola, rapeseed, or double-zero rapeseed meals for growing pigs. Studies lacking ingredient composition data or oilseed meals with EE > 6.5 % of DM were excluded. Chemical composition variables were first evaluated for multicollinearity and then subjected to stepwise selection for inclusion in the meta-regression. The meta-regression was performed using the MIXED procedure in Minitab, with ME as the dependent variable and selected composition variables as fixed predictors, while study was considered a random effect. Model adequacy was verified using the 'studentized' residuals and residual's normality. Outliers were identified as data points with residuals outside the (-3,3) range. The average chemical composition (% of DM) of the meals was: CP = 41.8 % ± 2.6, NDF = 34.2 % ± 7.31, ADF = 21.5 % ± 5.5, EE = 2.9 % ± 1.4, and ME = 2975 ± 317 kcal/kg of DM. The first model identified NDF, EE and ash as the main predictors of ME. However, ash was excluded due to collinearity with NDF ($r = 0.279$; $P < 0.05$). The final model revealed a negative effect of NDF (-25.31 kcal / % point of NDF; $P < 0.001$) and a positive effect of EE (60.4 kcal / % point of EE; $P < 0.05$) on ME. The R^2 of the final model including the random effect of study was 87.7 %. These results confirm the negative effect of NDF on ME, which is consistent with previous findings in the literature. The model highlights NDF as a key determinant of the energy value of canola meal and provides a practical predictive tool for estimating ME from routine chemical analyses.

Dietary former food products modulate fecal microbiota and are associated with improved carcass composition in heavy pigs

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Former food products (FFPs) from the food industry represent a sustainable alternative to cereals in pig nutrition. While their nutritional value has been documented, their impact on gut microbial ecology and its potential relationship with carcass traits remains unclear. This study evaluated the effects of increasing dietary inclusion of FFPs (0%, 20%, 40%) on fecal microbiota composition in heavy pigs and explored associations with performance and carcass characteristics. Thirty-six pigs were fed iso-caloric and iso-proteic diets containing 0% (Control), 20% (FFP20), or 40% (FFP40) FFPs in the diets of grower (20-60 kg BW), finisher I (60-100 kg BW) and finisher II (100-140 kg BW) pigs. Growth performance and carcass composition (dual-energy X-ray absorptiometry) were recorded. Fecal samples (n = 36) were analyzed by whole-metagenome sequencing, and the differential gene expression (DEG) analysis of jejunum mucosa was performed with RNA-sequencing. Feed efficiency improved linearly with increasing FFP inclusion (up to +9% in the finisher II period; $P < 0.001$), while carcass fat decreased (−6%; $P \leq 0.009$) and protein content increased (+3%; $P < 0.001$) compared with Control pigs. Alpha diversity indexes (Observed ASVs, Shannon, Evenness-Simpson) were not affected by diet ($P > 0.10$), indicating preserved microbial richness and evenness. However, beta diversity (Bray–Curtis) showed a tendency toward dietary clustering (Adonis $P = 0.079$), with FFP20 exhibiting the most distinct microbial profile. Linear discriminant analysis effect size (LDA > 3) identified 33 discriminant taxa. FFP20 showed the highest number of microbial markers, mainly belonging to the class Erysipelotrichia (including Turicibacteraceae and Turicibacter bilis). FFP40 was characterized by enrichment of Romboutsia (including Romboutsia timonensis) and members of Chlamydiaceae, whereas Control pigs were enriched in Bifidobacteriaceae (genus Pseudoscardovia) and Megasphaera. Core microbiota analysis (≥0.1% relative abundance; ≥80% prevalence) identified 21 shared taxa between Control and FFP20, while FFP40 exhibited a reduced core (16 taxa), suggesting dose-dependent restructuring of stable microbial populations. Also, correlation analysis revealed a strong positive association between lean meat percentage and Peptostreptococcaceae and Romboutsia timonensis, whereas belly fat positively correlated with Bifidobacteriaceae. RNA-sequencing of jejunal mucosa tissue revealed a small number of significant DEGs (adj. $P < 0.05$, $|\log_2 \text{ fold change}| > 1$). Therefore, gene set enrichment analysis was performed using ranked $\log_2$ fold change values. Comparison of the FFP40 and Control showed enrichment of gene sets associated with the cell cycle, lipid, alcohol, and sterol biosynthetic processes (adj. $P < 0.05$). Additionally, comparison of the FFP20 diet with the Control revealed enrichment of gene sets involved in lipid biosynthetic processes, regulation of the immune system and RNA processing (adj. $P < 0.05$). Direct comparison between the FFP40 and FFP20 groups further identified enrichment of cell cycle and carboxylic acid catabolic processes (adj. $P < 0.05$). In conclusion, dietary FFP inclusion modulated gut microbial community structure without affecting overall diversity and was associated with improved feed efficiency and reduced carcass fat deposition. Accordingly, the enrichment of the gene sets involved in lipid processes was identified in jejunal mucosa with inclusion of FFPs. Moderate inclusion (20%) induced the most pronounced microbial differentiation, while higher inclusion (40%) reduced core microbiota stability.

Digestibility of mycoprotein derived from Paecilomyces variotii in growing pigs

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Mycoprotein derived from the biomass of filamentous fungi through controlled fermentation has emerged as a promising alternative feed ingredient. Filamentous fungi are well known for their ability to secrete a broad spectrum of enzymes, enabling them to degrade and assimilate a wide variety of complex substrates. Mycoprotein grown on low-value sidestreams has low land use and the potential to increase European protein production and contributing to a circular food system. In pig production, feed is the major contributor to the carbon footprint, and the use of feed-efficient pigs together with feed ingredients with a low carbon footprint, such as by-products, is often highlighted as an important strategy to reduce the environmental impact of pig production. In this study, dried milled mycoprotein from Paecilomyces variotii grown on sulfate liquor from pulp production was used to determine its total tract apparent digestibility (TTAD) of organic matter and energy, and the standardized ileal digestibility (SID) of crude protein and amino acids in pigs. The analyzed chemical composition of the P. variotii mycomeal showed a high content of crude protein (52.3% on a dry matter basis) and amino acids (35 g/kg DM lysine and 7 g/kg DM methionine), as well as a high content of crude fibre (27.5% on a DM basis). In the digestibility study, a total of 14 individually housed pigs were used and assigned to two experimental feeds: a protein-free basal feed, and a feed in which 30% of the basal feed was substituted with mycomeal from P. variotii (PV). The pigs were crosses between Yorkshire × Hampshire and weighed 34.3 ± 1.76 kg at the start of the experiment. They were fed the experimental feeds for 11 days, including seven days of adaptation, followed by three days of fecal collection. On day 11, the pigs were euthanized, and ileal digesta were collected. The digesta and feces were freeze-dried prior to analysis. Feed and digesta were analyzed for dry matter, crude protein (CP), titanium dioxide, and amino acids. In addition, feed and feces were analyzed for ash and gross energy. Digesta samples from pigs fed the protein-free basal feed were used to calculate basal endogenous losses, and the results showed values that corresponded well to previously published values (CVB, 2019). The TTAD of organic matter in PV was 65.4 ± 1.52%, and of gross energy, 63.7 ± 3.14%. The SID of CP, lysine, and methionine was 73.0 ± 2.24%, 75.6 ± 2.46%, and 72.8 ± 2.66%, respectively. These results indicate that P. variotii grown on sidestreams from the forest industry is a valuable source of protein with acceptable SID values in pigs. However, the high crude fibre content limits its TTAD of energy and organic matter, and processing methods that reduce cell wall content could improve energy utilization in pigs.

The impact of replacement imported protein sources with alternative domestic protein feeds in the feeding of fattener pigs on of their fattening, slaughter and economic value

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The most expensive ingredient in animal feed is crude protein. Currently, the main source of protein in feed for pigs kept in the European Union countries is post-extraction soybean meal derived from genetically modified plants, imported mainly from the USA, Brazil and Argentina. About 2.5 million tons are imported to Poland every year, with the price of the component rising steadily, which negatively affects the profitability of pig production. Many European Union countries are carrying out a number of measures to reduce GMO feed, especially soy, in the feeding of farm animals. Research is underway to replace soybean-derived protein with protein from seeds of domestic legumes such as peas, lupins or faba beans. The aim of the research is to evaluate the replacement of post-extraction soybean meal with high-protein plants grown in Poland in the diet of fattener pigs in terms of their fattening and slaughter value as well as production profitability. Peas, faba beans, sunflower meal and hemp and linseed cake were the main source of protein in the experimental group. The experiment was carried out on 60 fatteners of the Puławska breed and lasted 119 days, and the average age at slaughter for both groups was 184.90 days. The body weight on the day of slaughter ranged from 105 to 128 kg (control group) and 105 to 124 kg (experimental group). The study included an assessment of: fattening value, slaughter value (including detailed dissection of the 5 most valuable cuts) and the economic and commercial value of the carcass. Although the experimental group was characterised by lower daily gains, higher feed intake, and thus a less favourable feed conversion ratio, the cost of feeding these fattening pigs was PLN 3.21 significantly lowerr in the first 21 days of the experiment and PLN 41.69 lower over the subsequent 98 days of the experiment compared to the control group. Thus, the difference between the total costs of feeding (119 days – the entire experiment) was PLN 44.9 . It was also found that the carcasses of the fattened pigs in the experimental group had statistically significantly lower backfat thickness and higher loin 'eye' height and carcass leanness, which in turn resulted in higher weight of valuable cuts. There was no effect of feeding modifications on the economic value (calculated according to the EUROP classification) and commercial value (calculated according to the unit price of individual cuts) of carcasses and half-carcasses and the commercial value of the five most valuable cuts. However, statistically significant differences were shown in the profit from fattened pig production. The production of fattened pigs from the experimental group was characterised by a higher profit by PLN 56.79 than the production from the control group. Based on the analysis of the results, a positive effect of substituting protein from soybean meal with a mixture of domestic protein components, i.e. peas, faba beans, sunflower meal and hemp and linseed cake was demonstrated primarily on the profit from fattened pig production.

Insect protein as a sustainable replacement for soybean meal in diets for growing pigs: effects on growth performance, plasma metabolites and gut inflammation

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Research on insect protein in pig diets has expanded rapidly since the 2010s, although insects have long been part of natural animal diets and were occasionally used in traditional feeding systems. Our aim was to evaluate alternative protein sources, specifically proteins derived from two insect species (Tenebrio molitor and Hermetia illucens), as potential sustainable substitutes for soybean by assessing their effects on growth performance, plasma metabolites and intestinal inflammation. A total of thirty (Duroc × Pietrain) × (Landrace × Large-White) pigs were randomly assigned to three experimental groups: a control group fed soybean meal and two groups in which 100% of soybean protein as the major protein source was replaced with insect protein either from Tenebrio molitor or Hermetia illucens. Diets were isoproteic and isocaloric, meeting the animals' nutritional requirements and formulated according to the ideal protein concept. Pigs were individually housed under temperature-controlled conditions (22 ± 1 °C) with ad libitum access to feed and water and were weighed weekly. Initial and final body weights were 17 ± 0.8 kg and 62 ± 2.4 kg, respectively. Animals were slaughtered 10 weeks after the beginning of the trial, after 24 h fasting, by electrical stunning and immediate exsanguination. Samples of faeces from the rectum were collected for calprotectin analysis. Additionally, blood samples were processed to determine biochemical parameters (albumin, β-hydroxybutyrate, HDL-cholesterol, LDL-cholesterol, total cholesterol, creatine, glucose, lactate, triglycerides and urea). Treatment effects were assessed by analysis of variance with a significance level of 0.05. No significant differences were found for feed intake among groups (1556, 1611 and 1485 g of dry matter/day, for the control, Hermetia and Tenebrio groups, respectively; SEM = 64.4; $P > 0.05$) indicating no differences in palatability of experimental diets. No significant differences between control and insect-fed groups were found regarding average daily gain, although insect-fed groups differed (848, 926 and 749 g/day, for the control, Hermetia and Tenebrio groups, respectively; SEM = 34.9; $P < 0.01$). There were significant differences for feed use efficiency. The highest value was for the Hermetia group, followed by the control and the Tenebrio groups (0.545, 0.575, 0.504; respectively; SEM = 0.005; $P < 0.001$). Regarding plasma biochemical metabolites, no significant differences were observed ($P > 0.05$), except for albumin, which was lower in the Hermetia group, compared with the control and Tenebrio groups (31.1, 36.3 and 38.3 g/L, respectively; SEM = 0.97; $P < 0.001$). Likewise, there were no differences in the levels of faecal calprotectin (1.99, 1.50 and 1.23 ng/g, for the control, Hermetia and Tenebrio groups; SEM = 0.47; $P > 0.05$). The results indicate that complete replacement of soy protein did not negatively affect growth performance, plasma biochemical parameters and markers of intestinal inflammation enhancing the sustainability of pig production without compromising animal performance. Funded by the EU project Facilitating Innovations for Resilient Livestock Farming Systems, abbreviation: Re-Livestock (reference number: 101059609).

Circular protein ingredients from fungal fermentation and blue mussel cultivation: ileal digestibility in broiler chickens

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Feed production is a major contributor to the environmental impact of animal-based food systems through greenhouse gas emissions, land use, and nutrient losses. In the Baltic Sea region, eutrophication caused by excess nutrient inputs remains a critical environmental challenge. Circular feed ingredients that recycle nutrients from industrial sidestreams and marine ecosystems may contribute to more sustainable animal production. Modern broiler production has evolved through genetic selection to produce high-yielding animals with excellent feed efficiency. These birds require high levels of digestible amino acids which today to a high extent originate from traditional protein sources like soybean meal. Globally, feed production accounts for 50-60% of broiler production's environmental impact, with soybean meal (15-25% diet inclusion) contributing significantly due to production and transportation. Novel circular highly digestible protein sources could partially replace soy thus improving the environmental performance of broiler production. The objective of this study was to evaluate the nutritional value and apparent ileal digestibility of novel circular protein ingredients in broiler chickens. A digestibility trial was conducted with 192 Ross 308 broiler chickens allocated to four dietary treatments. A conventional wheat–soybean meal diet served as control, while experimental diets replaced 20–25% of the control diet with blue mussel (Mytilus spp.) meal, Neurospora intermedia mycoprotein, or Paecilomyces variotii mycoprotein. The mycoproteins were produced through fermentation-based processes using food and industrial by-products, whereas mussel meal was sourced from cultivated blue mussels in the Baltic Sea. All ingredients represent locally produced, circular feed resources with low land-use requirements. According to analysis all test ingredients exceeded 30% crude protein (CP) on a dry matter (DM) basis. Mussel meal had the highest CP content of approximately 65%, and lysine and methionine contents of 49.3 and 15.4 g/kg DM, respectively. P. variotii had approximately 50% CP and lysine and methionine contents of 31.9 and 7.2 g/kg DM. Corresponding figures for N. intermedia was 30% CP and 15.9 and 4.8 g/kg DM content of lysine and methionine, respectively. Apparent ileal digestibility of organic matter (OM), CP, and amino acids was determined. All experimental diets showed CP and amino acid digestibility values comparable to the control diet, with some exceptions. Among individual amino acids, leucine and aspartic acid displayed significant differences ($P<0.05$), with digestibility coefficients for P. variotii being higher than those for N. intermedia. A significant difference ($P<0.01$) was observed in the OM, with control diet having the highest digestibility coefficient and P. variotii the lowest. In conclusion, fermentation-derived mycoproteins, particularly P. variotii, and mussel meal demonstrated promising CP and amino acid levels as well as favourable digestibility characteristics in broiler diets. These findings support their potential role as circular, low-impact feed ingredients contributing to more sustainable and resilient poultry feed supply chains.

From Winery By-Product to Functional Feed Additive: Polyphenol-Enriched Natural Deep Eutectic Solvents on Antioxidant Defense and Gut Morphology in Meat-Type Quails

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The increasing environmental impact of food and agro-industrial waste represents a major global concern. Among extensively cultivated fruit crops, grape (Vitis vinifera L.) produces large amounts of pomace as the primary solid byproduct of winemaking. This residue is a valuable source of bioactive compounds, such as polyphenols, with recognized health-promoting properties. To recover these high-value molecules, a sustainable green extraction approach based on Natural Deep Eutectic Solvents (NaDES) was applied starting from grape pomace (cv Sangiovese) collected in Apulia Region of Southern Italy, in accordance with the principles of green chemistry and circular economy. A NaDES-based edible and ready-to-use choline chloride/ascorbic acid (ChCl/AA) (2:1; mol/mol + 40% water) polyphenolic extract, previously characterized for its chemical composition and biological activity, was used as feed supplement. Thus, this study aimed to evaluate the effects of a natural feed supplement on the productive performance, serum components, oxidative status and gut histomorphology of growing quails. A total of 120 unsexed 7-day-old growing Japanese quails were randomly assigned to three experimental groups (n = 40 per group), with each group further divided into four replicates (10 per replicate), up to 42-days of age. The control group received a basal diet without additive, while the experimental groups were supplemented with ChCl/AA NaDES (DES) or polyphenol-enriched NaDES (E-DES) at a level of 0.3% diet (3 g/kg diet), respectively. The results indicated that dietary treatments did not significantly affect growth performance, meat nutritional composition, or the majority of serum biochemical parameters in quails. Notably, supplementation with polyphenol-enriched NaDES enhanced the redox status of growing quails, as evidenced by a significant improvement in serum lipid profile parameters, increased total antioxidant capacity (TAC), superoxide dismutase (SOD), and glutathione peroxidase (GPx) activities, and reduced malondialdehyde (MDA) concentrations compared with the other groups. Furthermore, quails receiving the E-DES diet exhibited significantly improved antioxidant markers in breast muscle tissue, including higher SOD, catalase (CAT), and GPx activities, along with lower MDA levels. Intestinal histomorphological analysis, a key indicator of gut health, revealed that E-DES supplementation significantly increased villus height and width, suggesting enhanced absorptive capacity. In conclusion, these findings support E-DES as an innovative and sustainable feed additive that enhances antioxidant status and intestinal morphology without impairing productive performance. Furthermore, green solvent extractions offer a strategic approach to valorizing agro-industrial by-products, converting waste streams into high-value natural bioactive supplements in line with circular economy principles and sustainable poultry production.

In vitro large intestinal fermentation of Iberian and Landrace pigs fed lentil, chickpea and soybean diets

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Locally grown lentils and chickpeas can replace soybean in pig diets, but information on their in vitro fermentation compared with soybean is limited. In addition, the diet of the donor pig may influence the fermentation capacity of the inoculum used in in vitro assays. This study evaluated the effect of donor pig diet on intestinal fermentation and the fermentative characteristics of lentils and chickpeas compared with soybean. Twenty-four (Duroc × Pietrain) × (Landrace × Large White) pigs (14 ± 0.3 kg) were allocated to three dietary groups: a control diet with soybean meal and two diets in which 50% of soybean protein was replaced by local legumes (culled chickpeas or lentils). Diets were isoproteic and isocaloric and met animal requirements. Pigs were individually housed at 22 ± 1 °C with ad libitum access to feed and water. After 10 weeks, pigs were slaughtered (66 ± 0.9 kg) and rectal faeces were collected and frozen (−20 °C) until incubation. On the incubation day, samples were thawed at 5 °C and pooled (four pools of two pigs per diet: soybean, lentil, chickpea). Bottles containing substrates (soybean, lentil, chickpea) were incubated at 39 °C for 24 h in duplicate using inoculum prepared from rectal content of each diet group.Data were analysed using a mixed model including substrate (soybean, lentil, chickpea), donor pig diet (soybean, lentil, chickpea) and their interaction as fixed effects, and donor pig (inoculum) as a random effect. Gas production, short-chain fatty acids (SCFA) and ammonia were measured. Incubations and analyses followed Pardo et al. (Animals 2023 13:1102).Donor pig diet did not affect 24 h gas production (852 ± 42 µmol/g; $P > 0.10$). Inoculum from pigs fed lentil diets showed greater dry matter disappearance than soybean, with chickpea intermediate (57, 54, 53% ± 1.0 for lentil, chickpea, soybean; $P < 0.05$). Ammonia-N concentration was higher with lentil than soybean diets (254, 298, 253 mg N/L ± 9.2 for soybean, lentil, chickpea; $P < 0.01$). Total SCFA production was greater with lentil than soybean diets, with chickpea intermediate (3760, 4719, 4289 µmol/g ± 186; $P < 0.01$). The same pattern occurred for acetate (2921, 3522, 3291 µmol/g ± 143; $P < 0.001$). Butyrate was highest with lentil, followed by chickpea and soybean (753, 647, 539 µmol/g ± 36; $P < 0.01$). Propionate did not differ among inocula (175 µmol/g ± 36; $P > 0.10$).Regarding substrate effect, lentil and chickpea fermented more than soybean (868, 1105, 584 µmol/g ± 42 for soybean, lentil, chickpea; $P < 0.001$). Chickpea showed greater dry matter disappearance than soybean, whereas lentil was lower (56, 49, 60% ± 1.0 for soybean, lentil, chickpea; $P < 0.001$). Ammonia-N concentration did not differ among substrates (267 mg N/L ± 9.2; $P > 0.10$). Chickpea produced the most total SCFA, followed by soybean and lentil (4170, 3614, 4984 µmol/g ± 186; $P < 0.001$). Acetate was lowest with lentil, with no differences between soybean and chickpea (2694, 3368, 3671 µmol/g ± 143; $P < 0.001$). Butyrate was highest with chickpea, followed by lentil and soybean (409, 621, 909 µmol/g ± 105; $P < 0.001$). Propionate did not differ among substrates (175 µmol/g ± 36; $P > 0.10$). In conclusion, donor pig diet did not affect gas production, but lentil diets increased SCFA, acetate, butyrate and ammonia compared with soybean diets. Chickpea showed the greatest fermentability, with higher SCFA production and dry matter disappearance.
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Sugar beets to support the ensiling of feed for pigs and poultry

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In the future, the safe and cost-effective storage of feed for pigs and poultry may become increasingly relevant. The vulnerability of supply chains means it is sensible to store larger quantities of feed directly on farms, ensuring that at least the current stock of animals can be fed until the end of the fattening period. Traditional storage capacities are rarely available in sufficient quantities nowadays and building them from scratch is very expensive. This exploratory study aims to produce mixed silage using washed and chopped sugar beets, as well as various types of grain, such as corn in the form of high moisture corn grain, wheat and rye, which can be easily, cheaply and safely stored in a bunker silo. For the silage trials, a total of approximately 1,000 tonnes of mixed silage, consisting of corn (C), wheat (W) and rye (R) each mixed with sugar beets (SBxC, SBxW or SBxR), was ensiled. The wheat and rye mixtures contained 30% sugar beets and 70% grain on a fresh matter basis. The high moisture corn grain mix contained approximately 15% sugar beets. A standard corn cob mill was used to grind the raw materials. The material was ensiled in a bunker silo. Once ensiling was complete, a representative sample of each silage was taken for analysis in the feed laboratory to determine its energy and nutrient content, as well as its ensiling quality. Following ensiling, the mixtures (SBxC, SBxW and SBxR) exhibited low pH values (3.71, 3.84 and 3.97), low ethanol content (g/kg DM: 1.96, 1.69 and 1.18), low viscosity (mPa·s: 1.35, 1.46 and 1.49), and low yeast (cfu/g: 3.5×10^3/–/0.5×10^3) and mould content ($<10^3$/–/$<10^3$). There was little difference in the levels of acetic acid (g/kg DM: 8.44/8.55/7.82), propionic acid (g/kg DM: 0.16/0.13/0.17) and n-butyric acid (g/kg DM: 0.13/0.37/0.37), nor in the levels of enzyme-soluble substances (g/kg DM: 952/939/936) or calorific value (MJ/kg DM: 18.7/18.6/18.6). Mixed silage made from sugar beets is an inexpensive feed for pigs. With the necessary technical adjustments, it could also be used for fattening poultry. The advantages lie in the high number of lactic acid bacteria, which can promote animal health when consumed. At the same time, this preservation method enables the cost-effective creation of a stable feed supply for monogastric animals using existing harvesting, processing and silage technology.

Common vetch seeds as home-grown protein feed for growing-finishing pigs

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Agricultural systems rely on amino acid supplying feedstuffs for monogastric animals. Home-grown protein feeds, like grain legumes, can contribute to circular systems and improve the resilience of agricultural systems with pig husbandry. Common vetch (Vicia sativa L.) has low demands on its habitat (Nguyen et al. 2020), so it can also grow on acidic soils and in dry regions. Its seeds contain high amounts of crude protein (up to 321 g kg^{-1} according to Chen et al. 2022) with higher proportions of methionine compared to other legumes (Seabra et al. 2001). However, feeding recommendations are scarce and the contained pyrimidine glycosides and cyanoalanine toxins might have a negative effect on palatability and health (Enneking 1994). The objectives of the study were to examine the use of diets for growing-finishing pigs with up to 20 % of grain legumes and to study the effects of raw vetch seeds of the variety Slovena (not specifically low in toxins; Höhne et al. 2025) on the performance of growing-finishing pigs. The first trial started in November 2025. A total of 80 pigs of a commercial breed were held on the organic experimental station of the Thünen Institute and fed a three-phase diet (growing phase 1: GP1 <50 kg body mass (BM), growing phase 2: GP2 50-75 kg BM, finishing phase: FP >75 kg BM) containing 29 (GP1+2) and 30 % (FP) grain legumes. In the control group (C), field peas and field beans were used as grain legumes and in GP1 49.5 % cereals and 18 % oilcakes, in GP2 57.2 % cereals and 11 % oil cakes, and in FP 64.2 % cereals and 3 % oil cakes were fed. In the vetch group (V), grain legumes were exchanged with vetch, which accounted for 10, 15, and 20 % of the diet in GP1, GP2, and FP, respectively. The other components were adjusted to gain equal energy and lysine concentrations between C and V (1 % less oil cakes in V). The feed was pelleted and supplied ad libitum. The health status (medical treatments) was continually monitored. Pigs and feed removals were weighed weekly. Pigs were slaughtered when weighing >119 kg. Paired t-tests were conducted to compare daily body mass gains (BMG) and feed conversion (FC) between both feeding groups. The pigs had mean BMG of 837 ± 52 g in GP1 and 1077 ± 72 g in GP2 and no significant differences between the feeding groups were observed. FC also did not differ significantly between the groups and was 2.2 in GP1 and 2.8 in GP2. Preliminary evaluations of 75 (37 C, 38 V) of the 80 pigs showed a mean BMG in FP of 1141 ± 62 g without significant differences between feeding groups. No feeding-related medical treatments were conducted. Previous studies have described that over 20 % raw common vetch seeds can be used in piglets (Seabra et al. 2001) and a low-toxin variety can be suitable in diets for growing pigs (Collins et al. 2005). The use of Slovena vetch seeds fed over the whole growing-finishing period of pigs in increasing amounts of 10 to 20 % of the diet did not impede growth or health in our first trial. To ensure that raw vetch seeds can be used safely in pig husbandry, examinations of carcass characteristics, meat quality, and blood parameters as well as consecutive trials are planned. Collins C, Dunshea F., Henman D, King R (2005) Australian J Exp Agric 45, 699-703. Höhne A, Machner M, Aulrich K, Bussemas R, Baldinger L (2025) Org Agr 15, 493–503. Nguyen V, Riley S, Nagel S, Fisk I and Searle IR (2020) Front Plant Sci 11, 818. Chen W, Wang Y, Lv X, Yu G, Wang Q, Li H, Wang J, Zhang X, Liu Q (2022) Int J Biol Macromol 216, 487-497. Enneking D (1994) Dissertation University Adelaide. Seabra M, Carvalho S, Freire J, Ferreira R, Mourato M, Cunha L, Cabral F, Teixeira A, Aumaitre A (2001) Anim Feed Sci Tech 89, 2-16.

Growth performance, nutrient digestibility, and nitrogen and phosphorus balance and excretion in fattening rabbits fed a diet primarily based on agro-food by-products

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Population growth and resource limitations will drive the transition of livestock farming, including rabbit meat production, from linear to circular value-added systems. In this context, replacing imported feeds with local protein sources and agro-food by-products can enhance the sustainability of livestock feeding. In this respect, the rabbit may be an ideal species for substituting food-grade feed materials with the aforementioned local resources in its diet due to its ability to efficiently digest high-fibre feeds. In fact, agro-food by-products are rich in dietary fibre. This study compared a conventional fattening diet (CF diet; alfalfa meal, soybean meal, wheat bran, sugar beet pulp, oats, and barley) with a fattening diet based primarily on local protein source and agro-food by-products (F-AFB diet; rapeseed cake, wheat bran, sugar beet pulp, corn distillers' grains with solubles, maize bran and flour, buckwheat hulls, and sunflower hulls), in which alfalfa meal, soybean meal, oats, and barley were completely excluded. Both diets had similar levels of crude protein (CP), neutral detergent fibre (NDF), acid-detergent fibre (ADF), limiting amino acids, and digestible protein. The F-AFB diet contained slightly higher acid-detergent lignin and ether extract, and slightly lower starch content. A total of 180 Hyplus rabbits (32 days of age) were allocated into two groups of 90 rabbits each and were fed either the CL diet or the F-AFB diet for growth performance evaluation. The fattening period lasted 42 days, after which the rabbits were 74 days old. In addition, total tract apparent digestibility (CTTAD) of the diets and nitrogen and phosphorus output were determined in 10 rabbits per treatment. No significant differences were observed in the final live weight of the rabbits (on average 2512 g), average daily feed intake (on average 135 g), or feed conversion ratio (on average 3.16). Neither mortality nor morbidity was observed during the entire fattening period, which represents an important finding of the study. Rabbits fed the F-AFB diet had significantly lower CTTAD of CP and starch, whereas CTTAD of NDF and phosphorus was significantly higher compared with the CF diet. Total nitrogen excretion and nitrogen retained did not differ between diets; however, the nitrogen retention coefficient was slightly lower in rabbits fed the F-AFB diet than in those fed the CF diet (0.40 vs. 0.45; $P = 0.042$). In contrast, phosphorus retained and the phosphorus retention coefficient were lower in rabbits fed the CF diet than in those fed the F-AFB diet (0.31 vs. 0.42 and 0.28 vs. 0.33, respectively; $P = 0.004$). Based on the obtained results, the F-AFB diet may represent a promising alternative to conventional feeding strategies. These findings support the concept of utilising local protein sources and agro-food by-products in rabbit nutrition and may contribute to the progressive integration of rabbit meat production into circular value-added feeding systems. This research was funded by the Ministry of Agriculture of the Czech Republic under grant QL24010136.

Faecal volatile fatty acids production in growing pigs fed local legumes partially replacing soybean meal as main dietary protein source

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Identifying alternative protein sources is a priority in pig production to reduce reliance on imported soybean meal. Local legumes such as peas, chickpeas and lentils are promising candidates due to their low environmental footprint, favorable nutritional profile and fermentable fiber content. The fiber fraction strongly influences hindgut microbial fermentation, producing volatile fatty acids (VFA) that may affect gut health and energy utilization. The aim of this study was to evaluate local protein sources (peas, chickpeas and lentils) as sustainable alternatives to soybean by assessing their effects on faecal fermentation and volatile fatty acid (VFA) production in a commercial pig breed. Thirty-two (Duroc × Pietrain) × (Landrace × Large White) pigs were randomly assigned to four groups: a control diet with soybean meal and three diets in which 50% of soybean protein was replaced by local legumes (peas, culled chickpeas or culled lentils). Diets were isoproteic and isocaloric, meeting animal requirements and formulated according to the ideal protein concept. Pigs were slaughtered after 10 weeks following a 24 h fast by electrical stunning and immediate exsanguination. Rectal faecal samples were collected and stored at −20 °C until VFA analysis. Briefly, 10 g of frozen faeces were acidified with 2 mL/g of 0.5 N HCl, vortexed and left for 10 min. A 2 mL aliquot was then centrifuged (16 000 × g, 15 min, 4 °C). Subsequently, 800 µL of supernatant were mixed with 500 µL metaphosphoric–crotonic acid and incubated overnight at 4 °C. Samples were centrifuged again under the same conditions, and the supernatant transferred to gas chromatography vials and stored at −20 °C until analysis. Acetate, propionate, isobutyrate, butyrate, isovalerate and valerate were quantified by gas chromatography using crotonic acid as an internal standard. Treatment effects were evaluated by one-way ANOVA ($P < 0.05$), with the pig as the experimental unit. No significant differences were observed in total VFA production (153, 139, 177, 153 mmol/L for control, chickpea, pea and lentil diets; SEM = 14.3; $P > 0.05$) or in the molar proportions of acetate (mean 0.672; SEM = 0.019), isobutyrate (0.020; SEM = 0.001), isovalerate (0.024; SEM = 0.002) and valerate (0.018; SEM = 0.002) ($P > 0.05$). However, butyrate proportion differed among treatments, being higher in pigs fed peas than in control and chickpea groups, with lentils showing intermediate values (0.102, 0.072, 0.072, 0.090, respectively; SEM = 0.084; $P < 0.05$). Although individual VFA differed, total faecal VFA remained similar among treatments. Higher butyrate proportions in pea and lentil diets may indicate beneficial effects on gut health when partially replacing soybean in growing pigs. Funded by the EU project Facilitating Innovations for Resilient Livestock Farming Systems, abbreviation: Re-Livestock (reference number: 101059609).

Effects of high-level inclusion of chopped and pelleted alfalfa on starter intake and growth performance in dairy calves.

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According to recent European and Canadian guidelines, calves are recommended to receive high milk allowances, often requiring a later weaning age. At the same time, commonly used high-starch starter diets are associated with an increased risk of ruminal acidosis and parakeratosis. Delayed weaning may therefore represent an opportunity to increase the inclusion of high-quality forage in calf diets. This study aimed to evaluate the effects of partial substitution of a conventional starter with alfalfa on feeding behaviour and growth performance in dairy calves. Eighty-eight Holstein × Jersey crossbred calves were enrolled at day 3 of age. Calves were blocked by body weight and breed composition, and assigned to one of the three dietary treatments: high starch starter (HS), starter including 30% chopped alfalfa (CA), or starter including 30% pelleted alfalfa (PA). Compared with HS, CA and PA diets differed in starch (35% vs. 25% DM) and soluble fiber content (5% vs 10% DM) while remaining isoenergetic and isonitrogenous. All calves were provided 20% of their body weight (BW) as whole milk from day 7 to 28, and were weaned at day 70 using a gradual step-down weaning approach. Calves were individually housed until day 77 and starter and milk dry matter intake (DMI) were recorded every two days throughout the study. Body weight was measured weekly and body measurements (heart girth, withers height and hip width) were collected on days 3 and 70. Age at first starter intake (AFI; ≥50 g DM/day) and gain-to-feed ratio (G:F; ADG/milk and starter DMI) were calculated. Data were analyzed using a mixed model with treatment as fixed effect, BW on day 3 as a covariate when relevant, and repeated measurements were modeled including age as fixed effect and calf as random effect. Milk intake did not differ among treatments. From day 49 to 70, starter DMI diverged among treatments with HS calves consuming 1.05 kg/d compared with 0.77 kg/d for PA ($P = 0.01$), while CA (0.93 kg/d) did not differ from either group. A similar pattern occurred for cumulative starter intake, such that calves consumed 42.5, 37.1, and 32.7 kg for HS, CA, and PA, respectively ($P < 0.01$). Differences in starter intake were confined to late pre-weaning phase, as no treatment effects were observed after weaning. At day 77, PA calves were lighter than HS (84.4 vs. 89.6 kg; $P = 0.04$), while CA (86.6 kg) did not differ from either treatment. Moreover, PA tended to have an older AFI than HS (29 vs. 24 days; $P = 0.07$) while a tendency for greater wither height variation was observed for CA compared with HS and PA (16.66 vs. 15.59 and 14.81 cm; $P = 0.08$). Milk intake, ADG, thoracic circumference, hip width, pre- and post-weaning G:F did not differ among treatments. Overall, differences consistently emerged between HS and PA; whereas, CA showed intermediate responses but did not differ from HS. These results suggests that pelleted alfalfa inclusion at a high level impaired starter DMI, growth, and feed efficiency. In contrast, the same alfalfa presented as chopped forage did not compromise performance, highlighting the importance of forage physical structure in pre-weaning diets. Further evaluation of long-term performance, nutrient digestibility, and gastrointestinal development will provide additional insight into the physiological processes driving these responses.

Nutrient intake and growth performance of Holstein calves supplemented with rumen-partially metabolizable methionine analog

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Methionine (Met) is the first limiting amino acid in young ruminants. Besides, low ruminal activity limits microbial protein synthesis, making the diet the primary source of Met. A rumen-partially metabolizable Met analog (HMBi) has been reported to improve body weight [BW] in calves, possibly due to a ruminal effect. However, few studies have been conducted, limiting clear recommendations for HMBi doses for calves. This study aimed to evaluate nutrient intake and growth performance of Holstein calves supplemented with HMBi during the pre- and post-weaning periods. Thirty-six male Holstein calves (7 ± 3 days of age; 40.26 ± 4.49 kg of BW and 9.2 ± 0.5 brix for serum total protein) were used in a randomized block design with three birth seasons as blocks, and supplemented with 0 (control), 8, or 16 g day^{-1} of HMBi. Within each block, one calf was slaughtered at 7 days of age, and another was fed a liquid diet exclusively throughout the preweaning period (a total of 42 animals). Calves were fed 8 L day^{-1} of milk replacer (12.5% solids), concentrate (ground corn, soybean meal, minerals, and vitamins [88.7% dry matter [DM]; 26.1% crude protein [CP]; 13.5% neutral detergent fiber [NDF]; 35.4% starch), and Tifton hay (81.2% DM; 10.1% CP; 75.6% NDF). Calves were weaned at 56 days of age using a gradual weaning protocol. After weaning, calves were fed a concentrate diet (84.8% DM; 21.2% CP; 13.3% NDF; 40.1% starch) and Tifton hay until they reached 126 kg BW. Concentrate, hay, and refusals were weighed daily to determine nutrient intake. Animals were weighed at the beginning of the study (7 days of age) and at every 7-day interval thereafter until slaughter (18 animals slaughtered at 56 days of age and 18 animals slaughtered at 126 kg BW). Data were analyzed as orthogonal contrasts to evaluate linear and quadratic effects ($P \leq 0.05$), using SAS 9.4. Initial age and measurements of calves fed milk replacer exclusively were tested as covariates ($P \leq 0.05$). The HMBi supplementation did not affect DM intake (DMI) of milk replacer (866.1 g day^{-1}) or hay (12.5 g day^{-1}), but linearly reduced ($P \leq 0.03$) concentrate DMI (120.9, 82.1, and 58.8 g day^{-1}), NDF intake (NDFI; 40.2, 35.0, and 32.08 g day^{-1}), and starch intake (61.2, 46.8, and 37.7 g day^{-1}) for 0, 8, and 16 g HMBi day^{-1}, respectively, at pre-weaning. The CP intake (CPI; 217.3 g day^{-1}), BW at weaning (61.1 kg), average daily gain (ADG; 0.354 kg day^{-1}), and gain:feed ratio (0.36) were not affected by HMBi doses during the pre-weaning period. At post-weaning, HMBi linearly increased (P=0.02) hay DMI (0.121, 0.151 and 0.163 kg day^{-1}) and reduced (P=0.03) concentrate DMI (2.46, 2.39 and 2.12 kg day^{-1}) for 0, 8, and 16 g HMBi day^{-1}, respectively, but did not affect total DMI (2.47 kg day^{-1}), NDFI (0.442 kg day^{-1}), starch intake (1.05 kg day^{-1}), CPI (0.55 kg day^{-1}), final BW (129.6 kg), ADG (0.98 kg day^{-1}), and gain:feed ratio (0.40). During pre- and post-weaning, the reduction in concentrate DMI with increasing HMBi doses may reflect greater metabolic satiety due to enhanced Met availability, a phenomenon supported by similar BW development in calves. Additionally, HMBi itself may limit intake by altering diet palatability, as observed in studies using amino acid sources in both solid and liquid diets. The greater the DMI, the more it can contribute to improved gastrointestinal development and health. The HMBi supplementation at levels up to 16 g day^{-1} reduced concentrate, starch, and fiber intake at pre- and post-weaning, without affecting body weight gain.

Ruminal fermentation and papillae development of calves supplemented with rumen-partially metabolizable methionine analog

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A high ruminal fermentation rate and the production of short-chain fatty acids (SCFA) can benefit rumen papillae development and increase digestive capacity in calves. Besides, the rumen-partially metabolizable methionine analog (HMBi) has been known to alter ruminal fermentation patterns in adult ruminants. This study aimed to evaluate ruminal fermentation and papillae development in Holstein calves supplemented with increasing doses of HMBi. Thirty-six male Holstein calves (7 ± 3 days of age; 40.26 ± 4.49 kg body weight [BW]) were used in a randomized block design with three birth seasons as blocks and supplemented with 0 (control), 8, or 16 g day^{-1} HMBi. Within each block, one calf was slaughtered at 7 days of age, and another was fed a liquid diet exclusively throughout the preweaning period (a total of 42 animals). Calves were fed 8 L day^{-1} of milk replacer (12.5% solids), concentrate (ground corn, soybean meal, minerals, and vitamins [26.1% crude protein [CP]; 13.5% neutral detergent fiber [NDF]; 35.4% starch), and Tifton hay (10.1% CP; 75.6% NDF). Calves were weaned at 56 days of age using a gradual weaning protocol. After weaning, calves were fed a concentrate diet (21.2% CP; 13.3% NDF; 40.1% starch) and Tifton hay until they reached 126 kg BW. Eighteen calves were slaughtered immediately after weaning, and the remaining 18 were slaughtered upon reaching 126 kg BW. At harvest, rumen contents were collected to determine the SCFA profile, pH, and NH3-N. Fragments (1 cm²) from the cranial and ventral sacs of the rumen were collected for morphometrics of ruminal papillae. Data were analyzed as orthogonal contrasts to evaluate linear and quadratic relationships ($P \leq 0.05$), using SAS 9.4. Initial age and measurements of calves slaughtered at the beginning of the study and those fed milk replacer exclusively were tested as covariates ($P \leq 0.05$). The HMBi doses did not affect total SCFA (83.13 mM), acetate (48.76 mM), propionate (38.57 mM), isobutyrate (1.02 mM), isovalerate proportion (1.26 mM), NH3-N (17.06 mg dL^{-1}), pH (5.99), number of papillae (NMP; 211), papillae area (MPA; 0.16 mm²), absorptive surface area (ASA; 3.46 mm²/mm²), papillae height (4.40 mm²) and width (2.24 mm²) and keratinized layer thickness (KLT; 14.70 µm) at weaning. Butyrate (7.76, 10.47, and 5.93 mM) and valerate proportion (2.31, 2.74, and 1.99 mM) showed a quadratic response for 0, 8, and 16 day-1 HMBi, at weaning. At post-weaning, propionate proportion reduced (45.44, 43.75 and 39.93 mM) and isobutyrate proportion (1.68, 2.31 and 2.70 mM), and ASA (14.03, 16.88 and 20.02 mm²/mm²) increased linearly, while butyrate proportion (5.86, 5.12 and 6.50 mM) and papillae height (5.43, 6.48 and 5.30 mm2) showed quadratic behavior for 0, 8 and 16 g day^{-1} HMBi. Rumen total SCFA (115.95 mM), acetate (43.07 mM), isovalerate (2.29 mM), and valerate proportion (3.56 mM), NH3-N (23.61 mg dL^{-1}), pH (5.95), NMP (97), MPA (1.80 mm²), papillae width (2.89 mm²), and KLT (29.87 µm) were not affected by HMBi doses at post-weaning. The greater butyrate concentration with 8 g day^{-1} HMBi at pre-weaning suggests enhanced carbohydrate fermentation and a greater potential for ruminal papillae development, as observed in ruminal ASA in the post-weaning period for doses of 8 and 16 g day^{-1} of HMBi, resulting in greater epithelial absorption. The feeding of HMBi at 8 g day^{-1} during the pre-weaning period and 16 g day^{-1} during the post-weaning period may maximize ruminal epithelium development of calves. Overall, feeding HMBi altered the ruminal SCFA profile during the pre- and post-weaning periods.

Hybrid rye as a forage source for Holstein cows around parturition

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Information on the use of hybrid rye as an alternative forage source to wheat straw in dry cow diets remains limited. Our first objective was to determine the effects of ensiling time (0, 30, 60, 90, and 150 d) on fiber composition of hybrid rye (KWS Progas, KWS Cereals USA, Champaign, IL) harvested at two maturities. Treatments were replicated once and assigned to one of two 4 ha plots of hybrid rye as follows: hybrid rye harvested at boot stage (BOO) and hybrid rye harvest at milky stage (MIL). Baled hybrid rye was transported by flat rack wagons (E-Z Trail Inc., Arthur, IL) from field to scale where each wagon weight was recorded. A total of 6 samples were removed directly from the bales using a forage sampler (Nasco Education, Fort Atkinson, WI) at their respective ensiling time then frozen for later analysis. For both objectives the statistical analyses were conducted using the MIXED procedure of SAS. At harvest, the average DM content for BOO and MIL was 16.9±0.01, and 29.0±0.06 %, respectively. Hybrid rye yield for BOO and MIL was 8.46 and 13.6 tons/acre, respectively. Rye silage in MIL had higher (P=0.002) concentrations of ADF (39.0%) than rye silage in BOO (34.0%). Rye silage in MIL had higher (P=0.03), concentrations of NDF (58.3%) than rye silage in BOO (54.8%). There was a treatment × time point interaction (P=0.03) in which rye silage in BOO had a lower concentration of starch at d 0, 30, and 60 (0.50, 0.75, and 0.15%, respectively) than rye silage in MIL (2.90, 2.70, and 1.85%, respectively). Rye silage in MIL had higher (P=0.04) concentrations of total acids (6.25%) than rye silage in BOO (4.19%). In conclusion, days ensiled negatively affected the nutritive profile and fermentation quality of hybrid rye harvested at milky stage but not for hybrid rye harvested at boot stage. Our second objective was to evaluate the effects of feeding hybrid rye baleage (KWS Cereals USA, Champaign, IL) from -32 ± 6 days relative to calving to 28 days in milk on performance of Holstein cows. Sixty-one multiparous pregnant Holstein cows, blocked by parity, previous 305-d mature-equivalent milk production, expected calving date, and BCS during the far-off dry period were assigned to 1 of 2 dietary treatments: total-mixed ration with wheat straw [(WS); 32.6% of dietary DM] or hybrid rye at the milky stage as the prepartum forage [(RM); 55.4% of dietary DM) in a randomized complete block design. After calving, half of the cows from each prepartum treatment group were assigned to a diet with alfalfa haylage (WSAH; RMAH; 25.2% of dietary DM) or a diet with hybrid rye at the boot stage (WSRB; RMRB; 13.2% of dietary DM) as the postpartum forage. Dry matter intake (DMI) and milk yield were obtained daily; body weight (BW) and BCS were measured weekly. During the prepartum period, there were no treatment effects observed for cows in WS and RM for DMI (12.3 and 12.5±0.24 kg/d, respectively; P=0.59), DMI as a percentage of BW (1.53 and 1.51±0.06%, respectively; P=0.84), BW (829.3 and 824.5±3.14 kg, respectively; P=0.25), BCS (3.54 and 3.54±0.04, respectively; P=0.93), or energy balance (2.54 and 2.50±0.61 Mcal/d, respectively; P=0.96). There was no effect of PRE (P=0.18) or POST (P=0.76) for milk yield. Cows in AH tended to have greater DMI (15.3±0.66 kg/d; P = 0.08) than cows in RB (14.1 ±0.66 kg/d) at week 1. Overall, hybrid rye harvested at the milky stage can replace wheat straw in controlled-energy dry cow diets without negatively affecting prepartum performance, and hybrid rye harvested at the boot stage can be included as a postpartum forage source without affecting early-lactation performance.

Carry-over effects of early postpartum protein supplementation on lactational performance, body weight, and nitrogen efficiency in dairy cows

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The objective of this on-going full lactation study was to investigate production and nitrogen-efficiency-related carry-over effects of supplemental protein provided to early postpartum dairy cows. A total of 41 Danish Holstein cows, of which 18 calving into their 2nd lactation and 23 calving into their 3rd or 4th lactation, were assigned to one of two treatments: 1) Control (lactating cow TMR consisting of % of dry matter (DM): 16.3% crude protein; 30.5% NDF, and 21.1% starch; n = 21), or 2) Boost [Control TMR + 4 kg/d of protein concentrate (42.3% crude protein, 7.9% NDF, 12.2% starch) during 1 to 7 days-in-milk (DIM), thereafter a gradual decrease by 200 g/d until DIM 28; n = 20]. After 28 DIM, all cows were fed the Control diet only. Data from calving up to 150 DIM were included in the current datasets. The protein concentrate was provided as a separate feed using the Roughage Intake Control System (RIC; Insentec BV) and the daily allowance for the Boost cows was adjusted according to DIM. All cows had ad libitum access to the basal TMR in RIC bins. Intakes of the protein concentrate and basal diet, as well as daily milk yield (MY) and body weight (BW) were recorded, and milk samples collected weekly from 2 milkings. The data were divided into two datasets with daily data for MY and DM intake (DMI) from DIM 1 to 28 and averaged weekly data from week 4 to 21. For energy-corrected milk yield (ECM), milk composition, and BW, data were average by week (week 1 to 21 of lactation). Data were analyzed using the lme function in R and the model included the fixed effects of treatment, DIM or lactation week, their interaction, and parity and the random effect of cow. The average concentrate intake for the Boost cows was 3.43 (±1.06) kg/d on DIM 1 to 7, and decreased on average 0.178 kg/d up to DIM 28. Overall, there were no difference between the treatments in total DMI during the first 28 DIM and up to week 14 of lactation, but Boost cows had around 1.6 kg/d greater ($P < 0.05$) DMI on week 15 to 18. Milk yield was on average 3.8 kg/d greater ($P < 0.05$) for the Boost cows across the first 28 d of lactation and remained 3.6 kg/d greater ($P < 0.05$; $P \leq 0.09$) after discontinuation of the protein supplementation on lactation weeks 4 to 11, 17, 18 and 21. Similarly, the Boost cows had or tended to have on average 3.7 kg/d greater ECM yield ($P < 0.05$; $P \leq 0.08$) across the 21 weeks of lactation. Milk true protein yield was around 10% greater on weeks 1 to 4 of lactation ($P < 0.05$), milk fat yield on average 11% greater on lactation weeks 4, 7, 11, 15, 19, and 21, and milk lactose yield on average 10% greater ($P < 0.05$) across the 21 lactation weeks for Boost cows. Milk urea concentration was 21 to 44% greater ($P < 0.05$) for Boost cows on lactation week 1 to 4, but was similar between the treatments thereafter. Nitrogen-Use-Efficiency was 8, 5, and 4 %-units lower ($P < 0.05$) for Boost cows on lactation week 1, 2, and 3, respectively, but did not differ between treatments across lactation weeks 4 to 21 being on average 35.9% (SEM = 0.95). Lastly, BW change did not differ between treatments. This study confirmed the potential of early postpartum protein supplementation to improve the lactational performance of dairy cows with positive carry-over effects up to at least 21 weeks of lactation. Further analysis will investigate the effects of the protein boost on nitrogen balance, and muscle and fat tissue mobilization. Future research should investigate the mechanisms behind the observed positive production response with carry-over effect, and potential role of individual AA in early lactation energy metabolism.

Impact of two levels of metabolizable protein and rumen-protected lysine and methionine for primiparous Holstein cows during the transition period and early lactation

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Efficient livestock production relies on accurate nutritional management and optimal replacement animal development. Heifers represent the future productive herd, and their growth, reproductive performance, and lifetime productivity are strongly influenced by early nutritional strategies. Proper evaluation of heifer development allows producers to ensure that animals reach appropriate body weight and physiological maturity at breeding, which is essential for improving fertility, longevity, and overall herd efficiency. Metabolizable protein (MP) is a critical component in ruminant nutrition as it represents the true protein available for absorption in the small intestine after ruminal digestion. Accurate determination of MP supply and requirements is essential to support growth, tissue development, and reproductive performance in heifers. Both insufficient and excessive protein supply can lead to reduced performance, inefficient nutrient utilization, and increased environmental nitrogen losses. Therefore, this study aimed to determine primiparous Holstein cows' performance when a standard MP (1,100 g MP/d) diet, without rumen-protected lysine (RPL) and rumen-protected methionine [RPM; Control; SMP]; a SMP diet with RPL [Kemin Industries Inc., Des Moines, IA; (0.54% RPL of DMI)] and RPM [Kemin Industries Inc., Des Moines, IA; (0.21% RPM of DMI); SMPAA], or high MP diet [(1,300 g of MP/d); HMP] were fed from -28 d to calving. Fifty-five primiparous Holstein cows were blocked by age at calving (months), dam PTA, sire PTA, expected calving date, and body condition score (BCS) during the far-off period and assigned to one of the three dietary treatments. Dry matter intake was recorded daily, and body weight (BW) and BCS were measured weekly. Prepartum energy balance (EBAL) was calculated weekly according to the NASEM (2021) equations. Colostrum was collected at the first milking after calving, weighed, and evaluated for IgG concentration using a colostrometer (Biogenics, Florence, OR) based on density correlation and Brix measurement using a digital refractometer (MISCO PA202x-400, Solon, OH). Colostrum samples were also chemically analyzed for fat content (ether extraction) and true protein (Kjeldahl method). Statistical analyses were performed using the MIXED procedure of SAS. Two treatment contrasts, SMP vs. SMPAA and SMPAA vs. HMP, were compared. Tukey's adjustment was used for the comparison of means. There were no differences between treatments or treatment by week interaction for DMI, BW, BCS, and EBAL ($P > 0.19$). No treatment differences were found for colostrum fat concentration, protein concentration, weight, IgG, or total solids $P > 0.18$. There was a tendency for cows in SMPAA to have a higher brix reading than cows in HMP (27.1 and 23.9, respectively; $P = 0.06$). Increasing metabolizable protein or supplementing RPM and RPL during the close-up period did not influence prepartum performance or colostrum quality. Evaluation of the subsequent lactation phase is needed to determine whether these treatments elicit carryover effects.

Effects of a multi-strain inoculant on alfalfa grass silage quality, digestibility, lactation performance and methane intensity in Holstein cows

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Maximizing forage quality in dairy cattle diets offers several benefits for animal productivity and efficiency, but depends on optimal forage management. The objective of this study was to evaluate a multi-strain inoculant on alfalfa grass silage (AGS) quality, fiber digestibility, lactation performance, and methane intensity. Alfalfa grass was harvested at first cut and ensiled in small-scale bunkers (19.8m x 6.1m x 2.0m) for 124 days. Fresh material was either untreated (CON) or treated with a multi-strain inoculant containing Pediococcus pentosaceous, Lentilactobacillus buchneri, and L. hilgardii at 2.74×10^5 cfu/g. Silage was sampled at weeks 0, 2, 4, and 6 relative to the feeding trial at two sampling heights (50 and 150 cm from the bottom of the bunker face). Samples were analyzed for fermentation profiles, enumeration of lactic acid bacteria, yeasts and molds. Fifty Holstein cows (111±23 d in milk; 2.9±0.9 lactations) were enrolled in a randomized, complete block design study with 1-wk covariate and 6-wk treatment periods. Diets contained 62% DM forage (41% AGS, 21% corn silage) and averaged 15.2% CP, 31.8% NDF, and 26.3% starch. Treatments consisted of untreated AGS (CON) and inoculated AGS (INOC). Cows were group-housed, fed individually 1x/d, milked 3x/d, milk sampled 6x/wk, and CH4 was measured with GreenFeed. Silage and cow data were analyzed using linear models including treatment, week, sampling height, and their interactions as fixed effects. Inoculated AGS had greater lactate and lower acetate concentrations than CON AGS at wk 2 ($P<0.05$) while total VFA concentration was not different between treatments and sampling height. Yeast counts in INOC AGS sampled at 150 cm decreased over time with lower counts at wk 6 ($P<0.05$) compared to CON. Aerobic stability at wk 2, 4, and 6 was improved ($P\leq0.05$) for INOC vs CON AGS at varying sampling heights. Independently of sampling height, ADF was lower in INOC AGS at wk 2 and 6, and aNDFom was lower in INOC AGS from at wk 0, 4, and 6 compared to CON ($P\leq0.05$). In-vitro fiber-digestibility (% NDF) was overall unchanged within treatments. Cows fed INOC AGS had greater DMI resulting in increased energy corrected milk, fat yield ($P=0.05$) and tended to increase true protein yield ($P=0.06$). CH4 production was unaffected by treatment, however, CH4 yield (g/kg DMI) and intensity (g/kg ECM) tended to be reduced ($P<0.07$) in the INOC diet. In this study cows consuming INOC AGS produced more milk as a result of improved silage aerobic stability and fiber composition while methane intensity tended to be reduced.

The effect of alternating concentrate crude protein supplementation on the milk production of spring-calving grazing dairy cows in Ireland

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Formulating lower crude protein (CP) concentrate feeds and increasing the dietary fermentable carbohydrate concentration is an effective means of reducing nitrogen (N) excretion from grazing dairy cows. The EU and Ireland have committed to climate neutrality by 2050, requiring a greater use of locally sourced feed ingredients with a lower carbon footprint, such as barley, oats and faba beans. In temperate grazing dairy systems, low CP concentrate supplementation in spring and autumn could potentially restrict milk production as grazing conditions are variable and grass silage is included in the diet. Native protein sources, such as faba beans, which are lower in RUP and AA, may exacerbate this. During these periods, including higher quality protein in the diet, such as SBM, may better support milk production. The objective of this experiment was to investigate 1) the effect of alternating concentrate CP supplementation strategies (CS) and 2) the effect of increasing dietary CP during periods of reduced grazed grass availability, on the milk production of spring-calving grazing dairy cows. Forty multiparous, spring-calving Holstein Friesian dairy cows were randomly assigned to one of two CS strategies for 39 weeks; 1) a 14 % CP concentrate (C14) formulated using barley, oats and faba beans or 2) an 18 % CP concentrate (C14S; C14 + 10 % SBM) in spring and autumn, and C14 during the main grazing season. Cows were 14 DIM when assigned to treatments. Cows were fed C14 or C14S from February for 6 weeks (P1) before being offered C14 during the main grazing season (27 weeks; P2), and returning to C14 or C14S from October until dry-off began (6 weeks; P3). Cows were offered a basal diet of perennial ryegrass (Lolium perenne) and rotationally grazed as one herd during the experiment. Grass silage was fed at an open feed face when required (125 g/kg DM CP, 462 g/kg DM NDF, predicted UFL = 0.87). Pre-grazing grass and grass silage samples were collected weekly. Mean pre-grazing pasture mass was 1398 kg DM/ha (± 394kg DM/ha). Milk yield was recorded daily and milk composition recorded weekly. Data were assessed for normality and analysed using the mixed procedure in SAS. Cumulative milk yield and milk solids yield were unaffected by CS strategy. Daily milk yield during the experiment was 26.9 kg/d and 26.2 kg/d for C14 and C14S respectively. Daily milk solids (MS; fat kg + protein kg) tended to be lower for C14S (- 0.08 kg/d; P = 0.082). In period 1, cows offered C14S had a significantly lower daily milk yield (- 1.2 kg/d; P = 0.031), energy-corrected milk yield (- 1.8 kg/d; P = 0.007) and MS yield (- 0.13; P = 0.006) than those offered C14. Milk production was similar between CS in period 2 and period 3. Alternating CS strategy via increased concentrate CP in the spring and autumn, coinciding with increasing proportions of grass silage in the diet, did not enhance milk production. Instead, cows offered C14S produced significantly less milk in spring, potentially due to an over-supply of RUP relative to requirements. Forage quality in this experiment was high, and this may have resulted in a greater dietary N supply than expected, impacting the results reported. This experiment shows that in high-quality temperate grazing systems, lower CP supplements (14 %) using native ingredients can support milk production as effectively as higher CP concentrates, potentially improving the N efficiency and reducing the carbon footprint of temperate dairy systems.

Effect of partial mixed ration allocation and pellet allocation in the automated milking system on performance of lactating Holstein cows

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Studies evaluating feeding management of cows in automated milking systems (AMS) largely focus on AMS pellet feeding strategies, despite the partial mixed ration (PMR) providing the greatest quantity of nutrients and influencing cow movement throughout the barn. This study evaluated whether feed allowance of the PMR interacts with amount of pellet provided in the AMS on dry matter intake (DMI), feeding and milking behaviour, reticular pH, and milk and milk component yields for Holstein cows. Eight Holstein cows in early lactation (68 ± 24 days in milk) were used in a replicated 4×4 Latin square design with a 2×2 factorial treatment arrangement. Treatments included PMR management where cows were provided PMR for ad libitum (AL; refusals equating to 10% as fed of the weight of the PMR offered) or slick bunk PMR management (SB; <3% refusals) in combination with a low (LA) or high (HA; 2 vs. 4 kg/d DM, respectively) pellet allocation in the AMS. Periods included 14-d for dietary adaptation and 7 d for data and sample collection. Cows were housed in a free-stall barn with individual feed bunks, allowing for measurement of feeding behaviour and intake in a feed-first guided-flow traffic AMS design layout. The AMS feed trough was modified to enable measurement of AMS pellet intake. Data were analyzed including the fixed effects of square, period, PMR management, pellet allocation in the AMS, and the interaction between bunk management and pellet allocation. Cow nested within square was the random effect. Providing the PMR AL tended (P = 0.08) to increase PMR intake (2.4 kg/d) over SB, while the pellet allocation did not affect PMR intake. Although HA cows refused 0.50 kg/d more pellet in the AMS than LA cows (P<0.01), HA cows had greater AMS pellet intake than LA cows (3.7 vs. 1.5 DM kg/d, respectively; P<0.01). Consequently, feeding PMR AL tended to increase total DMI over those fed PMR for SB management (31.3 vs. 29.0 kg/d, respectively; P = 0.08), and cows fed HA tended to have greater DMI than cows fed LA (31.3 vs. 29.0 kg/d, respectively; P = 0.09). Although no effect of treatment was detected for mean reticular pH, cows fed PMR for SB with the HA had the lowest minimum reticular pH (PMR ×AMS; P=0.02) relative to all other treatments. Likewise, cows fed PMR for SB with HA had the greatest duration and area of reticular pH <5.8 (PMR ×AMS; P<0.01) relative to the other treatments. While no effect of PMR management on milk yield was detected, yield tended to be greater for cows fed the HA when compared to the LA (61.9 vs. 58.7 kg/d; P=0.07). No treatment effect was detected for milk fat yield or concentration, but the de novo fatty acid (P=0.05) concentration tended to be greater for cows provided AL than SB PMR management (25.0 vs. 24.1% of fatty acids). Moreover, cows provided AL PMR had a greater concentration of mixed fatty acids than cows fed PMR for SB management (38.5 vs. 37.8% of fatty acids; P=0.04). These results indicate that for high yielding Holstein cows in early lactation, limiting PMR availability may increase risk for low ruminal pH when fed higher pellet allocations in the AMS. Moreover, feeding AL PMR may increase DMI and de novo and mixed fatty acids, while feeding more pellet in the AMS may also increase DMI and milk yield without affecting fat yield.

Effects of barley grain processing methods and dietary starch level on lactating dairy cows dry matter intake, ruminal fermentation, milk and milk component yields

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Barley grain is a common cereal grain used in Western Canada, Australia, and parts of Europe. As barley starch is rapidly degradable in the rumen, recommendations for barley grain processing focus on avoiding excessive production of fine particles. However, the impact of processing method may be more important when diets contain high starch than low starch concentrations. This study investigated whether processing method (ground (GB) vs. dry-rolled barley (DB)) and the dietary starch concentration (25% (LS) vs. 32% DM (HS)) affected dry matter intake (DMI), ruminal fermentation, milk yield, and milk composition for lactating Holstein cows. Eight cows (713 ± 80 kg BW, 162 ± 56 days in milk) were used in a 4×4 Latin rectangle design with a 2×2 factorial arrangement of treatments. Periods lasted 25-d and included 18 days for dietary adaptation and 7 days for data and sample collection. Data were analyzed using the mixed model of SAS including processing method, dietary starch, and their 2-way interaction as well as period included as fixed effects. Cow nested within square was included as a random effect. Dry matter intake was not affected by the processing method and starch level. Cows fed HS sorted against fine particles ($P < 0.01$) and sorted for long particles (19 mm sieve, $P < 0.01$) relative to those fed LS but there was no effect of barley grain processing method on feed sorting. Mean ruminal pH was greater for cows fed GB than those fed DB (6.23 vs. 5.98; $P = 0.03$). The duration that ruminal pH was <5.8 was greater for HS than LS (488 vs. 235 min/d; $P = 0.03$), and greater for DB than GB (510 vs. 213 min/d; $P = 0.02$). The concentration of total short-chain fatty acids and the molar proportions of individual fatty acids were not affected by treatment. Milk yield was not affected averaging 36.3 kg/d. Likewise, the concentration of protein and lactose were not affected by treatment. Milk fat concentration was reduced by nearly 0.5 kg/d (4.05 vs. 4.54%; $P = 0.03$) with HS than LS, but milk fat yield did not differ averaging 1.51 kg/d. Despite the reduction in milk fat concentration for HS than LS, there were no differences for the proportion of fatty acids arising from de novo, mixed, or preformed fatty acids. In conclusion, other than mean pH, the processing method had little effect on responses of cattle; while feeding HS increased feed sorting, reduced mean ruminal pH, and the concentration of fat in milk.

Dietary carbohydrate fractions as modulators of dry matter intake in high-producing Holstein dairy cows: a meta-analytic approach

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Dietary composition is a primary modulator of voluntary dry matter intake (DMI) in lactating dairy cows, yet the relative contributions of individual carbohydrate fractions remain incompletely characterised across production levels in large meta-analytic datasets. We hypothesised that neutral detergent fibre (NDF) and non-fibre carbohydrates (NFC) are the dominant dietary predictors of DMI, and that their associations with intake would differ across energy-corrected milk (ECM) yield classes. A database of 429 treatment means was assembled from 132 peer-reviewed studies published between 1984 and 2025, comprising 6,827 Holstein cows housed exclusively in freestall systems and fed total mixed (TMR) or partial mixed (PMR) rations. Only studies with completely randomised (CRD) or randomised block (RBD) designs were included; Latin square and crossover experiments were excluded. Treatment means were classified into three ECM classes: Class 1 (<30 kg/d; n = 113), Class 2 (30–40 kg/d; n = 174), and Class 3 (>40 kg/d; n = 142). Pearson correlations and multiple regression analyses were performed to quantify associations between dietary variables (crude protein [CP], NDF, acid detergent fibre [ADF], starch, NFC, and net energy for lactation [NEL]) and observed DMI, with ECM yield class and body weight (BW) as covariates. Data availability varied markedly across fractions: NDF was reported in 407 of 429 treatment means, starch in 232, and NFC in only 143; class-level NFC analyses were based on n = 50, 55, and 38 observations for Classes 1, 2, and 3, respectively, and should be interpreted with caution. Across the full dataset, NFC showed the strongest positive association with DMI (r = +0.39; n = 143), followed by starch (r = +0.26; n = 232), while NDF was the most consistently negative correlate (r = −0.27; n = 407). ADF (r = −0.14), CP (r = +0.10), and NEL (r = −0.03) showed weaker associations. Among animal-level predictors, ECM was the strongest (r = +0.58), followed by BW (r = +0.45), while BCS showed no association (r = +0.004). When stratified by ECM class, the NDF–DMI relationship was inconsistent (Class 1: r = −0.14; Class 2: r = +0.01; Class 3: r = −0.28), indicating that the overall negative relationship is mainly driven by cows at the extremes of the production range. The NFC–DMI association was positive in all classes and numerically strongest in Class 3 (r = +0.57), although the small sample sizes preclude firm conclusions at the class level. Multiple regression with ECM class and BW as covariates confirmed that NFC was a significant independent predictor of DMI (β = +0.20 kg DMI/% NFC; P < 0.001; n = 143), while NDF did not reach significance after adjustment (β = −0.05; P = 0.097; n = 407). These findings indicate that increasing dietary NFC supports higher voluntary DMI across production levels. The lack of an independent NDF effect after covariate adjustment suggests that its apparent negative correlation with DMI is largely mediated by production level and body weight. These results provide a quantitative basis for precision ration formulation aimed at enhancing feed efficiency and resource use in circular dairy systems.

Impact of feeder type on voluntary intake and milk production in lactating dairy cows

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The use of automated weighing feeders enables the continuous measurement of individual feed intake by recording the amount consumed at each visit, without restricting animals to a fixed individual feeder. While facilitating feed management and intake measurements, these systems may also alter feeding conditions and voluntary feed intake. This study compared voluntary intake and production performance of lactating dairy cows fed using shared automated weighing feeders or fixed individual feeders. Forty mid-lactation dairy cows were allocated to 4 balanced groups according to stage of lactation, parity, and intake and performance measured during a reference period. Cows were subjected to 2 feeding systems (weighing vs fixed individual feeders) while receiving the same diet composed of 65% corn silage and 35% concentrate, following a double cross-over design with four 2-week periods. The use of automated weighing feeders resulted in a higher dry matter intake (DMI; 27.0 vs. 25.3 kg/d; $P < 0.001$), whereas drinking water consumption at the trough was slightly lower (84.0 vs. 86.7 L/d; $P < 0.05$) compared with fixed individual feeders. Consequently, milk yield (33.0 vs. 32.0 kg/d; $P < 0.001$), milk fat content (41.5 vs. 40.8 g/kg; $P < 0.05$), milk protein content (33.6 vs. 33.2 g/kg; $P < 0.001$), and energy-corrected milk yield (34.2 vs. 32.6 kg/d; $P < 0.001$) were also higher. The higher intake observed with automated weighing feeders may be partly explained by greater freedom of access to the feed. In line with this hypothesis, the inter-daily variability of dry matter intake was higher with automated weighing feeders (2.32 vs. 1.54 kg; $P < 0.001$). In conclusion, automated weighing feeders increased voluntary feed intake and milk production in lactating dairy cows. These first results highlight the need to reconsider the evaluation of ad libitum DMI and the associated intake prediction models.

Quantifying energy expenditure in lactating dairy goats using the doubly labelled water method under pasture and indoor housing conditions

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Pasture-based systems (PBS) are widely regarded as beneficial for the welfare of ruminants, including goats. However, PBS may also elevate the risk of a negative energy balance in lactating dairy goats due to increased locomotion and higher maintenance energy requirements. The present study aimed to compare the daily energy expenditure (DEE) of dairy goats at peak lactation under a PBS with that of indoor-housed (IND) goats. In addition, the study evaluated whether measured DEE corresponds with established recommendations for the energy supply of lactating dairy goats. Eleven primiparous dairy goats (body mass: 43 ± 3.9 kg) were assigned to the two housing systems IND (n = 6) and PBS (n = 5). At the start of the experiment, goats were in early lactation (days in milk: 17 ± 10 days). Milk yield was recorded daily, and milk samples were collected twice per week. Daily energy expenditure was quantified using the doubly labelled water (DLW) method following established protocols over the course of seven days. The DLW method estimates daily energy expenditure in free-living animals by quantifying CO_2 production from the differential elimination rates of hydrogen and oxygen isotopes after administration of isotopically labelled water. This approach provides an integrated measure of whole-body energy expenditure under standard husbandry conditions. Milk samples were analyzed for gross energy content by bomb calorimetry. Because the DLW method captures energy expenditure for milk synthesis but does not account for the energy exported in milk, individual milk energy output was calculated and added to DEE to obtain the total energy balance. Group comparisons were performed using linear models with contrasts to account for unbalanced data. When assessing differences in total energy balance or calculated energy requirements between housing systems, body mass was included as a covariate. Statistical significance of housing system effects was evaluated using Type II ANOVA. Mean daily milk yield did not differ between housing systems averaging 2.2 ± 0.4 kg in IND and 2.3 ± 0.1 kg in PBS. Body mass was also comparable between both groups (IND: 43 ± 4.0 kg; PBS: 43 ± 3.8 kg). Total energy balance, i.e. DEE plus milk energy output was significantly higher in PBS (17.2 ± 1.3 MJ day^{-1}) compared with IND goats (15.9 ± 1.7 MJ day^{-1}, $p < 0.05$). Likewise, calculated energy requirements were higher in PBS (17.3 ± 0.9 MJ ME day^{-1}) than in IND goats (15.4 ± 1.7 MJ ME day^{-1}, $p < 0.05$). The ratio of measured total energy balance to calculated energy requirements did not differ between housing systems (IND: 104 ± 8%, PBS: 99 ± 9%), indicating that measured energy expenditure was only 4% higher than predicted in IND goats and only 1% lower in PBS goats, suggesting close agreement between actual and expected energy use across systems. Although milk yield and body mass did not differ between housing systems, total energy balance was higher in PBS goats due to higher milk energy contents. Furthermore, the comparable ratio of total energy balance to calculated energy requirements between both groups suggests that current energy recommendations already account for some of the system-specific costs of PBS.

Partitioning of vitamin E in lactating dairy cows at two levels of vitamin supplementation

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Vitamin E (VE) is a key lipid-soluble antioxidant involved in protecting biological membranes from oxidative damage and supporting immune functions. Supra-nutritional levels improved immune responses in some instances. We hypothesized that VE overfeeding could play an important role especially when heat stress occurs. The objective of this study was to evaluate the partitioning of VE to milk following the administration of two levels of VE in two seasons. Fourty-six multiparous Holstein cows (BW 750±78.8 kg) of a commercial dairy herd located in the Parmigiano Reggiano cheese-production area (N° of cows 883±48; milk yield 36±1.5 kg/d) were selected 7 days after calving, including 22 and 24 cows that calved during the winter (October to November) and summer (May to July) seasons, respectively. Cows assigned to two pens, balanced for parity and date of calving, received 2 hay-based total mixed ration (-TMR- aNDF 36 %DM, starch 27 %DM, CP 14.4 %DM, EE 2.6 %DM) supplemented with high (HVE; 8000 mg/d) or low (LVE; 1600 mg/d) doses of VE. Animals were observed from 7 to 150 days in milk (DIM). The girth circumference was measured individually at the beginning of the trial to estimate the BW and metabolic BW. Feed intake was measured weekly on a pen base by difference between the amount of feed delivered and orts. Meanwhile, the TMR of each group was sampled and chemically analyzed. Plasma samples were collected from the coccygeal vein at 7, 20, 60 and 150 DIM, and analyzed for the metabolic profile and VE content. Milk yield was recorded, and milk samples were collected individually 3 times per season during the milk samplings performed by the breeder association. Milk quality was determined by MilkoscanTM while VE content was determined using an iCheck fluorometer–spectrophotometer (iCheck Vitamin E; BioAnalyt GmbH, Teltow, Germany). Data were statistically analyzed by generalized linear models using SPSS software (IBM SPSS Statistics for Windows, Version 29.0; IBM Corp., Armonk, NY). An increase in the DMI was observed in the summer season in the HVE vs LVE group (29.2 vs 28.0; p=0.027), although metabolic profile, milk yield and quality were similar between groups. The highest daily VE secretion in milk was observed in the HVE group during the winter season (222 and 146, 133 and 105 mg/d, for HVE and LVE in winter season, HVE and LVE in summer season, respectively), while no differences were found in the LVE group between seasons. The plasmatic content of VE was higher in the HVE group (p<0.001), and especially in the winter season reaching 382 mg/d. A higher proportion of VE partitioned to the milk on the VE assumed was observed for the LVE group (p<0.001) especially in the winter season (7.71 vs 5.44 for winter vs summer; p<0.001), indicating a possible better absorption efficiency. The retention of VE was always higher in the HVE group (p<0.001), with the highest observed in the summer season (9109 vs 8981 mg/d; p=0.003). Similar results were found considering the proportion of VE retained on the assumed (93.7 vs 84.78 for HVE vs LVE in winter season, p<0.001; and 95.6 vs 89.06 for HVE vs LVE in the summer season, p<0.001).

Effect of whey or skim milk as the main protein source in milk replacer on growth performance in dairy calves

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Both whey and skim milk are considered valuable sources of protein in calf milk replacer (MR). However, whether MR containing protein derived from whey or skim milk should be preferred in the nutrition of dairy calves is still debatable. Thus, this study aimed to determine the impact of whey-based and skim milk-based MR on the growth performance of dairy calves. Ninety male and female Holstein calves were randomly allocated at day 10 of age to one of three groups (n = 30/group) and fed: 1) whey-based MR that contained no skim milk (G0); 2) MR that contained 25% skim milk (G25); and 3) MR that contained 50% skim milk (G50). In G0 and G25, whey protein concentrate was used as the main ingredient. Groups were balanced for the number of bull and heifer calves as well as calves born from primi- and multiparous cows. Prior to allocation to the study, calves were fed first colostrum (4 L) and subsequently a mixture of surplus colostrum and raw milk. Growth performance and health parameters of calves were monitored from 10 until 60 days of age. Experimental MRs were formulated to contain a similar concentration of crude protein (22% as is), crude fat (20%), Lys (2.3%), Met (0.75%), Thr (1.7%) and Trp (0.44%). From the first day of the study, the calves were fed 7.5 L daily of MR in three feedings (2.5 L/feeding), which was prepared to ensure 160 g of MR powder in 1 L of milk (in total 1200 g/day/calf). Starter mixture was offered ad libitum from the first day of the study. Feces were scored daily using a 4-point scale and all medical treatments were recorded. Passive immunity was determined between day 2 and 3 of age. Data were analyzed using the MIXED procedure in SAS. The statistical model included the effects of experimental group, calf sex, maternal status (primiparous or multiparous), and the interaction between them as classifying variables. Because the interactions were non-significant, they were removed from the model. When justified, initial body weight was included in the model as a covariate. Passive immunity and initial body weight (BW) did not differ between groups ($P \geq 0.24$). Cumulative MR and starter intake did not differ between groups ($P \geq 0.28$). Final BW was higher for G25 compared to G50 (83 vs. 79.8 kg; $P \leq 0.05$) but did not differ between G0 and G50 (81.9 kg) or between G0 and G25 ($P > 0.05$). Similarly, average daily gain and feed efficiency (gain-to-feed) were higher for G25 compared to G50 ($P \leq 0.05$) but did not differ between G0 and G25 or between G0 and G50 ($P > 0.05$). Fecal score and number of medical treatments did not differ between groups ($P \geq 0.09$). In the current study, the rearing outcomes of calves fed MR containing only whey protein did not differ from those fed MR containing skimmed milk powder. When skim milk powder inclusion in MR increased from 25% to 50%, this resulted in a lower final body weight by 8.5%. Using a milk replacer containing whey and a moderate proportion of skimmed milk powder (25%) resulted in the best growth performance of calves and the highest feed efficiency.

Improving milk yield while reducing diet carbon footprint in European lactating dairy cows.

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Soybean meal (SBM) is a major protein source in dairy cow diets worldwide, due to its high protein content and competitive price. Though, SBM raises environmental issues in European farming because of its high carbon footprint. Replacing SBM by European protein sources implies amino-acid balancing to maintain zootechnical performance. The objective of this trial was to assess the effects of SBM removal combined with essential oils, methionine and lysine supplementation in the diet of lactating dairy cows on milk yield (MY) and energy corrected milk (ECM) persistence. The trial was conducted in the experimental dairy farm of Agroevolution (France), composed of 105 lactating cows producing on average 38 Kg of milk per day (milk protein content = 34.5 g/kg, milk fat content = 44.5 g/kg), and fed a partial mixed ration based on corn silage, grass silage and concentrates. A total of 69 cows after peak lactation were selected and allocated to three groups of 23 lactating cows each, blocked by parity (15 multiparous and 8 primiparous cows per group), days in milk, and milk yield the week before the trial. Following a two-weeks adaptation period with all cows receiving a CONTROL diet, the cows entered a seven-weeks experimental period. The CONTROL group was fed the CONTROL diet, including 2.5 Kg of SBM. MET and MET-LYS groups were fed diets without SBM, replaced by rapeseed meal, rapeseed expeller and corn DDGS, and supplemented with essential oils to stimulate microbial protein synthesis (VALOPRO ACTION ®, 20 g/cow/day). All three diets were formulated to get the same net energy and crude protein content (1.6 Mcal/kg DM and 163 g/kg DM), but differed in methionine and lysine content due to rumen bypass methionine and lysine supplementation (digestible methionine intake of 63, 76 and 76 g/cow/day and digestible lysine intake of 191, 190 and 203 g/cow/day, in respectively CONTROL, MET and MET-LYS diets; NDS Professional ®). Individual feed intake and milk yield were recorded daily, while milk protein and fat contents were analyzed weekly. MY (resp. ECM) persistence was assessed by comparing the slopes of MY (resp. ECM) over time using a multivariate mixed model including parity, day (resp. week), group, group:day (resp. week), parity:day (resp. week), and a random effect, depending on cow, on intercept and slope (R software, nlme package). At the start of the experimental period, cows averaged 204 ± 105, 199 ± 83, and 182 ± 77 days in milk (DIM) in the CONTROL, MET and MET-LYS groups, respectively. MY at intercept did not differ among groups (39.8 ± 1.7, 37.5 ± 1.4 and 38.9 ± 1.6 kg, respectively in CONTROL, MET and MET-LYS groups, P=0.560), and was higher in multiparous than in primiparous cows (+4.8 kg/d, P=0.001). MY persistence was improved in both MET and MET-LYS groups, compared to the CONTROL group (+ 0.03 and + 0.06 kg/day, respectively, P<0.001), and was higher in primiparous cows than in multiparous cows (+0.03 kg/day, P<0.001). ECM at intercept did not differ among groups (39.9 ± 1.5, 38.5 ± 1.3 and 41.5 ± 1.4 kg in CONTROL, MET and MET-LYS groups, respectively, P=0.249), and was higher in multiparous than in primiparous cows (+4.7 kg, P<0.001). ECM persistence did not differ between groups (P=0.888) nor parity (P=0.238). Carbon footprints of MET and MET-LYS diets were 2.3 and 2.2 kg/cow/day lower when compared to CONTROL diet (-17 and -16%, respectively; Agribalyse® database). This study highlights the positive effects of methionine, lysine and essential oils supplementation on MY persistence when replacing soybean meal by local protein sources in lactating dairy cows' diet in a European context. These results support the hypothesis that replacing SBM in dairy cow diet may be sustainable in European dairy farms.

Environmental strategies and milk production in European dairy basins : focus on the climate smart french initiative

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Is the dairy dynamic in major production areas influenced by environmental regulations ? A review of the main production zones reveals a diversity of contexts and also variability depending on the issues at stake: from non-binding voluntary incentive schemes in France to production constraints in the Netherlands, or economic constraints via a tax in Denmark for example.In a first approach we will present the characteristics and environmental results of major dairy production countries in and outside Europe regarding the main environmental indicators : greenhouse gas emissions, water quality, air quality and biodiversity. The dairy basins studied will be USA (9 millions of head), New Zealand (4.6 millions of head), France (3,2 millions of head), Ireland (1,5 millions of head), Netherlands (1,5 millions of head), Denmark (0,5 millions of head) with for example GHG gross emissions from 0,77 to 1,3 kg eCO2/ liter of milk. The results and regulation analysis will be presented in this first part, in particular from following projects Dairy-4-Future, Interreg Atlantic Area (2018-2022); ClieN-Farms H2020 (2022-2026) Climate-neutral systems; PATHWAYS H2020 (2021-2027) Pathways for transitions to sustainability in livestock farming and food systems; Carbon farming Life Europe (2021-2027) Low-carbon approaches and carbon credits. In a second part we will analyse how environmental regulations and targets of major agro industries are affecting production and ambitions of the major dairy basins. Policys framework will be detailed from Nitrate and Habitats Directives to the global methane pledge and how they transcript for farming sector in the various dairy countries. Strategies of large dairy groups will be presented with objectives and resources deployed to encourage changes in practices. During the third part, a testimony of French dairy sector initiatives will be presented: research and tools/methods developed, advisory system, practices selected and implementation on dairy farms, incentives, barriers and farmers feedback, lessons learned and results for the dairy sector. The carbon footprint of milk in France has significantly decreased over recent decades. Between 1990 and 2010, emissions per litre of milk dropped by about 20–24%, reflecting major efficiency gains in dairy farming . More recently, progress has slowed, with about a 6-7 % reduction between 2016 and 2021 . Overall, the sector has already achieved substantial reductions but now faces diminishing returns. Several solutions have driven this improvement, with more 35,000 of farm assessment with CAP2ER LCA tool. Farmers have optimized herd management, increasing productivity per cow and thus reducing emissions per litre. Feed changes, such as replacing imported soy with local crops, have also lowered emissions. Better manure management and spreading techniques have reduced methane and nitrous oxide emissions. In addition, carbon storage in grasslands and improved energy efficiency in processing and transport have contributed. These combined actions explain the steady but now slower decline in the carbon footprint of milk in France.

Structural consolidation and sustainability priorities in the Polish dairy sector (2004–2026): lessons from a fast-transforming EU dairy region

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Poland is one of the largest milk producers in the European Union, with production increasingly concentrated in three major dairy regions—Podlasie, Mazovia, and Greater Poland—which together account for more than 50% of the national dairy cow population. Over the last two decades, the Polish dairy sector has undergone profound structural transformation and intensification. The number of dairy herds declined from approximately 751,000 in 2004 to fewer than 236,000 in 2022, with a further decrease between 2022 and 2023 (from 180,800 to 173,300 farms keeping cows). At the same time, average herd size increased from 3.9 cows in 2005 to more than 13 in 2020. Total milk production rose by about 60% despite an estimated 21% reduction in cow numbers, reflecting major gains in productivity, technological uptake, and management intensity. By June 2024, stocking density had reached 14.6 dairy cows per 100 ha of agricultural land, underlining the growing importance of nutrient cycling and manure management at both farm and regional levels. Nevertheless, assessing the structure of Polish dairy farming remains challenging: 2020 data indicate that medium-sized farms with 20–99 dairy cows accounted for about 21% of holdings, whereas large farms with more than 100 cows represented only about 1%. This case study shows how a consolidating dairy region can simultaneously increase output and retain scope for further development. The Polish dairy sector still has considerable growth potential, driven by ongoing productivity gains, investment in herd management and forage systems, and strong regional specialisation. Farms are also increasingly adopting automated milking systems and on-farm monitoring technologies, including milking robots, sensor-based health and production control, and precision data platforms. These innovations enhance management capacity, enable more targeted resource use, and may help lower environmental pressure through modern production technologies. However, future development will be shaped by two interrelated pressures: growing competition from external markets, including the possible effects of expanded trade with Mercosur countries, and the need to reduce methane emission intensity and nitrogen losses to water and air in response to increasingly stringent EU climate and environmental requirements. Against this background, we identify priority areas and transferable solutions aligned with the Sustainable Development Goals. These include precision feeding and forage-quality optimisation to reduce enteric methane emissions per kilogram of milk; whole-farm nutrient accounting supported by decision-support tools; innovations in manure handling, such as separation, covered storage, anaerobic digestion, and other context-specific mitigation options; and robust digital monitoring and reporting systems to enable credible low-carbon product claims and participation in emerging incentive schemes, including carbon-related mechanisms. Strengthening advisory services and cooperative models will be essential to accelerate uptake while preserving economic viability across farms of different sizes. In Poland, such advisory support is provided mainly by private-sector consultants associated with feed companies and by advisors linked to organisations representing cattle breeders and milk producers, rather than exclusively through state-organised extension services. Taken together, these developments provide a practical pathway for combining competitiveness, environmental performance, and resilience in a rapidly transforming dairy region.

Developments in the dairy sector in the Baltic States region: from recent trends to medium-term outlook

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The dairy sector in the Baltic States plays a strategically important role in regional food systems, rural employment, and export performance, while operating within the broader framework of the European dairy market. The sector is increasingly shaped by structural consolidation, technological modernization, environmental requirements, and geopolitical uncertainty. To position the Baltic region within the wider European context, the study first applies factor analysis and cluster analysis using Eurostat data grouped into three categories: dairy production capacity, market dynamics, and economic–demographic indicators. The results confirm substantial structural differences across Europe. Central and Western European countries exhibit higher efficiency and export orientation, whereas Eastern and Southern European regions, including parts of the Baltic area, face structural constraints and relatively lower competitiveness. These findings highlight the need for region-specific development strategies. Building on this regional perspective, the study conducts an in-depth medium-term assessment of dairy sector development prospects to 2032 using an integrated modelling framework that combines the AGMEMOD partial equilibrium model with the TOPSIS multi-criteria decision-making method. The AGMEMOD model was recalibrated using updated national data for 2015–2023, significantly improving its empirical accuracy: the projected milk yield for 2024 (8,124 kg per cow) closely approximates the actual statistical value (8,239 kg). In AGMEMOD two scenarios were developed: (A) Targeted and intensive farm modernization, and (A2) Limited farm modernization under conditions of policy uncertainty. Scenario A is defined by the integration of productivity-enhancing factors, taking into account the impact of such factors as the genetic progress, modern technologies i.e. precision feeding, robotic milking etc. These factors are not included directly, but as an estimation of their impact on the productivity of cows. Scenario A results in a 4.9% higher average milk yield compared to the baseline, while maintaining stable overall production without herd expansion, reflecting productivity-driven growth. In contrast, Scenario A2 shows slower productivity gains and increased vulnerability to cost and regulatory pressures. The TOPSIS evaluation identifies Scenario A as the most favourable alternative (proximity coefficient = 0.980), demonstrating that gradual and structured technological integration – particularly through precision herd management and digital monitoring systems – enhances sectoral resilience and competitiveness. Overall, the findings suggest that sustained technological modernization, supported by adaptive and flexible policy instruments, is critical for maintaining productivity and market performance in the Baltic dairy sector. And the integrated AGMEMOD-TOPSIS framework offers a robust methodological tool for evidence-based policy evaluation and strategic planning, contributing to the broader scientific discourse on regional dairy development pathways within the European and global context.

Recent Developments in Dairy Farming in Slovenia and Croatia

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Slovenia and Croatia entered the EU in, respectively, 2004 and 2013. The developments in Slovenia are very much based on the principle in the former Yugoslavian Republic that private farmers were allowed to have a max of 10 ha agricultural land. Also, a high share of agricultural land is owned by people who moved to the city, which results in a carpet of many parcels. For that reason, till to-day, most dairy cows (about 70 %) are still held inside for efficiency reasons, although grazing is encouraged. The family farm is the standard with most common 20 to 70 cows. All of family supports the farm. Statistical data show that the correlation between family income and size of farm is weak. Especially the management of farm determines the income. Milk production level of the 80% recorded cows in Slovenia is 7,674 kg/year with 4.06 % fat and 3.41 % protein. Of the total cow herd, 39% is Holstein-Friesian, 33% Simmental, 7.6% Brown Swiss and 20.4% are crossings. In Croatia, the proportion of medium sized herds (from 10 to 30 cows) is dominant (39.40%), while relatively large farms with more than 100 cows represent around 13% of all dairy farms. The Simmental breed is prevalent in milk production and is reared mostly on family farms. Holstein-Friesian cows represent the second most important cattle population and are kept mainly on enterprises specialized in milk production. In Simmental breed, milk production of recorded herds is 7,157 kg/year with 4.10 % fat and 3.50 % protein. Holstein-Friesian breed has 8,838 kg/year with 4.20 % fat and 3.50 % protein. Milk prices tend to be a bit higher in Croatia than in Slovenia, where the milk is mainly sold and transported to Italian companies. For comparison, the prices are in between the Netherlands (high) and Lithuania (low). Focus in Slovenia is on special products and direct sales, employing family labour. About 10 % of the dairy farms engage in those on-farm activities. Because of large Nature 2000 areas and less favourable areas, the environment, especially nitrogen application, has been for longer time a focus field. Also, because in the valleys with fertile soils, dairy farming has become quite intensive. Mechanization and automatization is popular. In Croatia, emphasis is more on efficiency and farm size, imitating Western European countries. In both Croatia and Slovenia, societal discussions about the animal's sectors are rather intense, mainly from the viewpoint of animal welfare (tie-stalls, outside conditions, calf rearing). Case farms in both Croatia and Slovenia will be presented showing strategies and future aspirations of the respective farmers/ families.

The Ukrainian Meat and Dairy Sector and Agricultural Science under Wartime Conditions: Current Challenges, Adaptation Mechanisms and Development Prospects

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Livestock production represents a strategic component of food security and rural economic stability in Ukraine. Since the onset of the full-scale war in 2022, the Ukrainian meat and dairy sectors have operated under severe disruption affecting farm infrastructure, supply chains, labour availability, veterinary services, and access to domestic and international markets. These challenges have created a highly unstable production environment and have significantly undermined the sustainability of livestock enterprises. This contribution analyses the main challenges currently affecting livestock production systems in Ukraine and identifies the key adaptation mechanisms implemented by producers and agricultural research institutions to maintain production continuity under wartime conditions. Special attention is paid to the role of scientific support and knowledge transfer in strengthening sectoral resilience. The analysis considers implications for food security and continuity across broader livestock supply chains. Wartime conditions have substantially affected the operational management of livestock farms. Disruptions in feed supply, electricity shortages, logistical constraints, and rising production costs have negatively influenced herd productivity, reproductive efficiency, and animal health management. Dairy cattle and pig production systems, two of the most important branches of Ukrainian livestock production, have been particularly exposed to these pressures. At the same time, volatility in milk and meat prices, reduced investment capacity, and uncertainty regarding market access have created additional structural risks for the long-term development of the sector. Despite these constraints, Ukrainian livestock producers have demonstrated considerable adaptive capacity. Farms have introduced a range of management strategies aimed at maintaining productivity and reducing risks, including the optimisation of feeding systems, reinforcement of biosecurity measures, improved herd health monitoring, and more efficient reproductive management. Increased collaboration among farmers, veterinarians, and research institutions has become an essential element of crisis response and knowledge exchange. Agricultural science plays a key role in supporting the sector under these conditions. Ukrainian research institutions continue to develop applied solutions in the fields of reproductive biotechnology, disease control, animal genetics, and sustainable livestock production. In addition, strengthened international scientific collaboration and the use of digital communication platforms have made it possible to continue research and advisory activities despite wartime limitations. The Ukrainian experience highlights the importance of scientific support, adaptive farm management, and international cooperation in sustaining livestock production systems during periods of severe socio-economic disruption. The lessons derived from this experience may contribute to the development of more resilient animal production systems and provide valuable insights for the European livestock sector in the face of growing global uncertainty.

Description of risk factors and management practices associated to mastitis and milk quality on organic dairy farms across the USA

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Mastitis is a costly disease that affects profitability and cow welfare. In the USA, the use of antibiotics is prohibited, in contrast to European organic farms, where their use is restricted but not strictly banned. Therefore, in both scenarios, it is essential to identify management practices to prevent mastitis so we can improve and enhance the future development of a growing organic dairy sector by improving milk quality and farm profitability, with practices that can be applied across diverse organic systems globally. The objective of this study was to describe risk factors and management practices and find associations to clinical mastitis (CM) incidence and bulk tank somatic cell score (BTSCS) on organic dairy farms across the USA. Through farm visits, we surveyed 82 organic farms in 12 states and evaluated milking systems. Farms were categorized by region as West (18%), Southwest (4%), Midwest (54%), Southeast (2%), and Northeast (22%). Farms were also categorized by size as small (<100 cows, 60%), medium (100 to 1000 cows, 28%), and large (>1000 cows, 12%). The average reported annual CM incidence was 12 ± 7 in 100 cows-year. The median milking herd size was 75 cows [range: 13, 4081]. The average daily milk yield was 23 ± 6 kg/cow/day. We found that 86% of farms overmilked their cows, including 53% using manual cluster removal. Also, only 4% of farms had optimal pulsator settings, 31% had unstable vacuum during milking, and 59% of farms performed milking system evaluations once per year or less. Our dataset had 85 variables. Dimensionality reduction was performed using principal components analysis and stepwise variable selection. Principal components (PC) and selected variables were used to build 4 models to explain variables associated with BTSCS and CM incidence. Region and farm size were forced into all models as covariates. For the first 2 models, where we used the first 2 PC (34% explained variance), we did not find any associations between the PC and BTSCS nor CM incidence. For the other 2 models, where we used the selected variables, milk yield (+0.5% ± 0.2/kg), rewarding employees for good milk quality (+7.7% ± 2), CM identification (red swollen quarter: -6.5% ± 3), and cluster removal method (automatic: -4.6% ± 3) were associated to CM incidence. Moreover, "bulk tank somatic cell count threshold of concern" (+0.006 ± 0.0001), the importance of employees in mastitis control (-0.2 ± 0.08), and the use of vaccines to prevent CM (+0.27 ± 0.13) were associated with BTSCS. In conclusion, perception and attitudes about mastitis, CM and milking system management explained most of the variability of our data and are associated with mastitis incidence and milk quality. One practical application of these results is that dairy farm advisors should emphasize frequent milking system evaluations.

Economic opportunities through shorter dry periods for Swiss dairy cows

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The health and fertility of high-yielding dairy cows are key determinants of biological andeconomic efficiency in dairy production. Shortening the dry period in multiparous cows hasbeen suggested as a management strategy to improve robustness, fertility, and profitability.This study evaluated the economic implications of shortened dry periods in Swiss dairysystems, focusing on milk yield, fertility, health-related costs, and farm-level profitability. The analysis was based on 12,012 lactations from Holstein cows evaluated by HolsteinSwitzerland. Lactations with dry periods of 30 and 60 days were compared regardingstandardized 305-day milk yield, fertility parameters (first insemination success, N3 index,number of inseminations per pregnancy, and delay time), and expected costs of relevanthealth disorders. Contribution margins were calculated for three production levels (9,000,10,000, and 11,000 kg milk per cow per year).Cows with a 30-day dry period produced approximately 100 kg more milk over 305 days thancows with a 60-day dry period. Fertility also improved: first insemination success increasedfrom about 70% to 80%, the N3 index declined from 10% to 5%, inseminations perpregnancy decreased from 1.5 to 1.3, and delay time was reduced from 45 to 35 days.Health-related costs were consistently lower with the shorter dry period.Although a shorter dry period increased basic feed costs due to a longer lactation period, thiswas more than compensated by lower costs for replacement, supplementary feeding, fertility,and health. Overall, contribution margins increased by CHF 69 to 150 per cow per year,depending on production level.These results suggest that shorter dry periods may represent not only an economicopportunity at farm level, but also a promising strategic option for Swiss dairy production.Under commercial farming conditions, this approach could support a better balance betweenproductivity, animal health, fertility, and sustainability, and may also be relevant for dairyregions facing similar challenges.

Iodine in the dairy chain: from measurement to farm management, product characterization and consumer knowledge

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Milk and its derived products are a cornerstone of human iodine intake, yet the levels of this essential micronutrient vary widely, reflecting the complexity of factors across the production chain. Ensuring a consistent supply of iodine begins with reliable analytical measurement and requires a holistic feed-to-fork perspective, encompassing farm management, industrial processing, and dietary consumption. In respect to the analytical framework, a fundamental first step is accurate measurement. "We can improve what we can measure": only with reliable and accessible analytical tools can the sector aim to standardize iodine content, enhance it where needed, and monitor products efficiently. Advances in rapid and cost-effective methods may offer new opportunities to guide decision-making throughout the chain. At farm level, feeding strategies and dietary supplementation, constitute the primary determinant, establishing a direct link between intake and milk content. Farm management practices, such as the use of iodine based teat disinfectants, may further contribute to transfer. Downstream, processing operations including pasteurization, skimming, cheese making, brining, and ripening, further shape the distribution and retention of iodine across diverse dairy products. All these dynamics generate notable variability among dairy matrices, from raw and commercial milk to cheeses, ricotta, and yogurt, likely affecting their contribution to recommended daily intake. In this scenario, consumer knowledge of dairy foods' role as a primary iodine source remains limited, emphasizing the importance of education and communication to fully leverage their nutritional potential. Addressing these challenges requires an integrated approach. By combining precise measurement, informed feeding and management practices, detailed product characterization, and enhanced consumer understanding, the dairy chain can maximize its contribution to human nutrition. Such a strategy not only supports public health but also reinforces the value of dairy products as reliable carriers of essential micronutrients in food systems. In this perspective, improved iodine management and clear product characterization may provide additional tools to differentiate dairy products and strengthen their positioning in the market as reliable dietary sources of iodine. A consistent and well-documented iodine content could support the use of nutrition claims on product labeling, while increased consumer awareness of their nutritional value may foster more informed choices, ultimately enhancing the attractiveness and overall perception of the dairy sector and its products. This research was funded by Bando 2022 PNRR Prot. P20228RP2Z "The relevance of iodine in human nutrition: filling the gap from the farm to the fork".

Association between calving interval, milk yield and early post-partum mortality among Swedish dairy cows

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Calving interval (CInt) is a key management parameter in dairy herds, traditionally targeted at 12–12.5 months. Extended CInt has been reported to improve fertility without compromising milk yield, and have been suggested to benefit cow health by reducing exposure to the high-risk periparturient period. However, retrospective studies have associated prolonged CInt with increased mortality risk around calving. One proposed mechanism is that extended late lactation with low yield may lead to excessive body condition at calving and subsequent metabolic disorders. Cow mortality affects both farm profitability and consumer trust in the dairy sector. Given the rising mortality rates in high-producing dairy systems, clarifying the associations between milk yield and mortality around calving is therefore warranted. The objective was to study associations between CInt, milk yield and on-farm mortality within 30 days after third calving in Swedish dairy cows. Data from the Swedish Official Milk Recording Scheme included 139,444 cows with second–third CInt recorded between 2015 and 2022. Calving interval was categorized as <11.7, 11.7–13, 13–15 and >15 months. On-farm mortality was defined as euthanasia, unassisted death or slaughter on farm within 30 days postpartum. Milk yield was classified as second-lactation 305-day ECM (<9,000, 9,000–11,000, 11,000–12,000, >12,000 kg) or categorized into three classes: intermediate (±2 kg from herd average), high (>2 kg above), and low (>2 kg below). Associations were analyzed using logistic regression (SAS PROC GLIMMIX), including breed, CInt and yield class as fixed effects and herd as a random effect. Overall, 2,482 cows (1.78%) died within 30 days after third calving. No interaction between CInt and yield class was detected. CInt >13 months was associated with increased mortality risk ($p<0.05$), with the highest risk at >15 months. High-producing cows (>12,000 kg ECM) and cows above herd average at last insemination also had higher mortality risk. CInt and milk yield were associated ($p<0.001$): longer CInt was linked to higher 305-day yield, but to lower yield relative to herd average at last insemination, reflecting late-lactation decline. In a subset of particularly high-producing herds (n=150, 18,115 cows), no significant associations between CInt, yield and mortality were detected. Overall mortality was slightly lower (1.65%) than in the full dataset (1.8%). This may indicate that herd-level management in high-producing herds may mitigate potential adverse effects of prolonged CInts. Potential contributing factors may include superior dry cow and transition cow management, better reproductive decision-making and more targeted selection of cows for reinsemination, or other herd-level factors. These findings highlight opportunities to reduce mortality in practice through improved herd management and advisory strategies. High-performing herds may also have a more homogeneous population of high-yielding cows, potentially facilitating more consistent management decisions compared with the broader population. In conclusion, prolonged CInt in second lactation was associated with increased on-farm mortality within 30 days postpartum, particularly when exceeding 15 months, although this risk appeared less pronounced in high-yielding herds.

Automatic lameness detection in dairy cows using a limb-specific weighing platform

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Lameness is one of the most common and economically relevant health problems in dairy cows and remains a major challenge for animal welfare, labour efficiency, and sustainable herd management worldwide. Automated detection systems therefore represent an important field of development in precision livestock farming and may support dairy producers in improving health monitoring and management decisions. The aim of this study was to investigate whether lameness and/or claw disorders can be automatically detected using a limb-specific weighing platform. For this purpose, 54 dairy cows on two dairy farms in the canton of Lucerne (Switzerland) were weighed on a limb-specific weighing platform for 300 seconds each. Prior to weighing, each cow was assigned a locomotion score (LS) according to Sprecher et al. (1997). Within two days after weighing, functional claw trimming was performed by a professional claw trimmer. Claw disorders were documented according to the ICAR Atlas using the software "KLAUE", and a cow claw score (CCS) was calculated according to Sarbach et al. (2025). These data were expanded with existing datasets from comparable studies. In total, data from 337 dairy cows on eight farms were analysed using descriptive and statistical methods. To minimise bias caused by very heavy or very light animals, six weight parameters were defined to quantify asymmetric weight distribution, of which the three most suitable were included in the statistical models. A statistically significant association was found between LS and weight distribution, as well as between CCS and weight distribution, indicating that limb-specific weight distribution may serve as an objective indicator for automated claw health monitoring. However, the platform was only partly suitable for reliable lameness detection under practical conditions. The best-performing model achieved a sensitivity of 74%, meaning that 26% of lame cows were misclassified as healthy, while most other models identified fewer than 50% of lame cows. Specificity was considerably higher, ranging from 70% to 99%. The study also revealed a relatively high lameness prevalence compared with other studies, with approximately 25% at the animal level (n = 336) and 61% at the farm level (n = 42). These findings suggest that limb-specific weighing platforms are not yet sufficiently accurate as a stand-alone diagnostic tool for routine on-farm use, but they represent a promising basis for future early warning systems in commercial dairy production. In combination with additional animal-based indicators and claw health data, such systems could contribute to earlier intervention, improved herd management, reduced labour demand, better animal welfare, and lower lameness prevalence. Although this study was conducted in Switzerland, the approach is relevant for dairy farming more broadly and may support the transition towards more efficient and sustainable production systems in different dairy regions.

Cow hygiene and bedding microbiology in compost-bedded pack dairy systems with and without organic substrate

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Compost-bedded pack (CBP) housing is increasingly adopted to improve cow comfort. However, bedding design and management may influence microbial dynamics, cow hygiene, and exposure to mastitis-associated microorganisms. We hypothesized that the inclusion of an organic substrate in CBP would be associated with lower and more homogeneous bacterial loads and improved cow cleanliness under commercial conditions. The objective of this study was to microbiologically characterize and compare two CBP systems used in dairy farms in the central basin of Córdoba Province, Argentina: CBP with added organic substrate (CB-S) and CBP without substrate (CB-W). Two commercial farms were evaluated. Composite bedding samples and milk samples from lactating cows were collected for microbiological analysis, and cow hygiene was assessed using a standardized visual scoring system across anatomical regions. Bacterial counts were $\log_{10}$-transformed prior to analysis and differences between systems were evaluated using analysis of variance ($p<0.05$). Environmental and opportunistic bacteria commonly associated with bovine mastitis were detected in both systems, including coagulase-negative staphylococci, Escherichia coli, Enterococcus spp., Corynebacterium spp. and Bacillus spp. Bedding from the CB-W system showed significantly higher bacterial loads than CB-S (8.58 ± 0.55 vs. 7.65 ± 0.09 $\log_{10}$ CFU/g; $P = 0.0182$) and greater bacterial diversity. Cows housed in CB-S showed significantly better hygiene scores than those in CB-W (1.05 ± 0.12 vs. 3.01 ± 0.10; $p< 0.0001$), with consistent differences across anatomical regions. These results indicate that bedding design influences both microbial conditions and animal cleanliness in CBP systems. The use of organic substrate was associated with more stable bedding conditions and improved hygiene, highlighting management strategies that may support udder health and contribute to One Welfare outcomes in dairy production systems.

Under construction – an approach to building animal welfare expertise in vocational training

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Expertise in animal welfare is essential for farmers in order to meet growing political and social demands for high animal welfare standards. To achieve these standards, intrinsic motivation among animal handlers and specialised knowledge in animal welfare is required. This expertise includes knowledge about the animals, practical skills in handling, and an empathetic attitude toward animals. Embedding this expertise in vocational training can promote animal welfare–oriented competence within the agricultural sector. The project 'Tierschutzkompetenz' [animal welfare expertise in vocational training of farmers], conducted by the Thünen Institute of Organic Farming, Institute of Vocational Education and Adult Education (IfBE) at Leibniz University Hannover (LUH) and the Agricultural Education Center Echem (LBZ), aimed to strengthen animal welfare competence in education through the development of teaching units and learning materials. As a first step an online survey gathered responses from 340 farmers in Lower Saxony who were registered as agricultural instructors for cattle, pigs, or both. They were asked about their understanding of animal welfare, the welfare tools they use on their farms, the training provided to agricultural apprentices regarding animal handling and transportation and their preferences for further education on animal welfare. The results showed that farmers perceive animal health as more important than the emotional state of the animal or the possibility to engage in natural behaviour. The instructors identified calmness and patience as the most important factors for successful interaction with animals. Accordingly, the handling of animals and low-stress stockmanship skills are considered important topics for further educational training. Existing data from farm management tools is used frequently for animal welfare management. Only about one-third of the respondents use special tools based on animal welfare indicators. Nine training units were developed for vocational training in cattle and pig husbandry. Their aim was to raise awareness and promote a balance between the three concepts of animal welfare: emotional state, animal health, and natural behavior. By teaching basic concepts of animal welfare, the training aims to enable participants to address animal welfare issues and actively participate in professional discussions on this topic. Existing units on animal welfare monitoring have been revised to help trainees to develop an eye for the animals by familiarising them with animal welfare indicators. One training session each for cattle and pigs teaches the basics of handling animals during transport. In order to further strengthen the anchoring of animal welfare in agricultural education and training, it is particularly important to improve cooperation between the various learning venues: the agricultural farm, the vocational school, and educational training centers. This cooperation is the focus of the follow-up project 'TiKoPLUS – animal welfare, competence and cooperation between learning venues'. 'TiKoPLUS' is funded as an InnoVET PLUS project by the Federal Ministry for Education, Family affairs Senior citizens, Women and Youth, granted by the Federal Institute for Vocational Education and Training (BIBB), (grant number 21IVP054C). The project 'Tierschutzkompetenz' was funded by the Federal Ministry of Food and Agriculture (BMEL) based on a decision of the Parliament of the Federal Republic of Germany, granted by the Federal Office for Agriculture and Food (BLE), grant number 2820MDT122.

The use of chlorophyll extract as post dipping in the photodynamic inactivation of microorganisms

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According to the United Nations' 2030 Agenda, one of the sustainable development goals is to ensure sustainable consumption and production patterns. The need to ensure food safety includes, in addition to microbiological risks, concerns about antimicrobial-resistant bacteria. Mastitis is an inflammation of the mammary gland that directly affects milk production, generating economic losses and compromising the health of animals. In addition to reduced production and loss of mammary gland functionality, mastitis represents a risk to public health, as pathogenic microorganisms and their proteins can be transmitted through milk. Given these concerns, there is a growing interest in natural alternatives for the prevention and control of mastitis, effectively minimizing the use of antimicrobials. Photodynamic Inactivation of Microorganisms (IFDMO) is a treatment focused on the interaction between a photosensitizing compound and light of appropriate wavelength, which leads to the formation of highly reactive species capable of eradicating different strains of microorganisms. The present study explored the photodynamic efficiency of Chlorophyll Extract nanocapsulated in micellar copolymer Pluronic® F127 in post-dipping applications aimed at preventing subclinical mastitis and the quality of the milk produced was evaluated via somatic cell count (SCC). Chlorophyll was extracted from spinach (Spinacia oleracea). The bactericidal activity of chlorophyll extract encapsulated in F127 was evaluated against Staphylococcus aureus, and the MIC was determined in the concentration range between 13.6 and 0.1 mg mL-1. The analysis of microorganism growth determined an MIC of 6.8 mg mL-1 and a MBC of 13.6 mg mL-1. The Extract at a concentration of 3.40 (mg/mL) was incorporated into a copolymer matrix (F127). The experiment was carried out in the dairy cattle sector of the Iguatemi Experimental Farm (FEI) belonging to the State University of Maringá (UEM). The solution was applied to the teats of eight Holstein animals (Cross over) after milking and irradiation with the red LED source for 30 seconds. The control was carried out with 1000 mg mL-1 iodine solution. Milk collections were carried out on days 7, 14, 21 and 28. For the milk samples obtained, there was no statistically significant difference in the total aerobic mesophilic count when comparing the application of Chlorophyll Extract and the Control (iodine). Regarding the milk composition, the levels of the evaluated parameters are in accordance with the minimum standards required by current legislation IN 76/77 (BRAZIL, 2018). There was no difference between treatments in relation to Somatic Cell Count (SCC) $p>0.05$. The values of somatic cells were in accordance with the regulations in force in Brazil (5.36 log10 iodine and 5.35 log10 chlorophyll extract). The results obtained demonstrate the feasibility of using IFDMO with chlorophyll extract in post-dipping applications, guaranteeing the quality of the milk produced, since Photodynamic Inactivation is recognized for not generating bacterial resistance, being a promising alternative to the use of conventional antibiotics.

Integrative Characterization of Feed and Water Microbiomes in Dairy Production Systems

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European dairy production has intensified toward larger farms, improving productivity but raising concerns about biosecurity, animal welfare, and sustainability [1]. Forage acts as both a nutrient source and a microbial reservoir, while drinking water, despite constant animal contact, remains underexplored [2].So far, studies have characterized individual compartments using short-read sequencing but rarely integrated feed-water systems or examined the effects of farm size [3].To address this gap, we characterized the microbiomes of 18 Portuguese dairy farms, categorized by size and selected based on biosecurity level.We aimed to establish a foundational understanding of feed/water microbiomes and generate actionable knowledge to improve management, enhance biosecurity, and sustain dairy systems. We tested: (H1)forage type is a primary driver of bacterial, fungal, and viral communities in feed; (H2)biosecurity and water system cleaning frequency as determinants of pathogen load and diversity in drinking water; and (H3)feed-water sharing a core set of potentially pathogenic taxa, suggesting microbial links across farm compartments. Samples were collected from feed bunks (50g) and water troughs (250 ml filtered with 0.22 µm filters). Total DNA extracted and sequenced using Nanopore long-read technology. Low DNA yield prompted amplicon sequencing of water samples and shotgun metagenomics of feed samples. Taxonomic profiles were generated using Epi2ME; functional annotation was performed using EggNOG-mapper/FunFun for fungi and Prokka/PICRUST2 for bacteria. Clostridium and Lactococcus dominated bacteria, Pichia and Cutaneotrichosporon fungi in water;while Acetobacter and Lactobacillus dominated bacteria, and Pichia and Saccharomyces fungi in feed. Feed microbiome was characterized by low diversity,while water exhibited greater richness. Feed and water shared 20% of the total microbial taxa and 80% of the potential pathogenic organisms detected, including Aspergillus flavus and Klebsiella pneumoniae. A higher pathogenic load in feed samples was detected in farms with lower biosecurity levels.In water samples, this pattern was found in farms with infrequent cleaning of water troughs. Forage type and water-trough cleaning frequency emerged as the strongest microbial management levers in dairy systems.Despite high taxonomic turnover, functional profiles remained broadly conserved, with feed enriched in fermentation pathways and water exhibiting enhanced nutrient cycling activity, indicating ecological redundancy that stabilizes dairy farm microbiomes under changing management conditions.The shared pathogen core between feed and water justifies integrated monitoring programs via ONT multi-domain profiling. These findings establish actionable ecological benchmarks linking microbiome management to animal health, productivity, and dairy biosecurity across production scales. Clay, N., et. al., Dairy intensification: Drivers, impacts and alternatives. Ambio,2020.Manzocchi, E., et al., Feeding cows with hay, silage, or fresh herbage on pasture or indoors affects sensory properties and chemical composition of milk and cheese. J Dairy Sci, 2021. Perdomo, A. and A. Calle, Assessment of microbial communities in a dairy farm from a food safety perspective. Int J Food Microbiol.

Immunomodulatory Effects of Bacterial Extracellular Vesicles from Wild and ATCC Escherichia coli and Staphylococcus aureus: A Comparative In Vitro Study

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Bovine mastitis remains one of the most significant challenges affecting dairy production, with Escherichia coli and Staphylococcus aureus being among the most prevalent causative agents. Beyond bacterial colonization, extracellular components such as bacterial extracellular vesicles (BEVs) may contribute to host inflammatory responses and tissue damage. This study compares the effects of wild isolates and reference ATCC strains of E. coli and S. aureus on cell death and inflammatory responses in vitro. BEVs are isolated from field strains obtained from mastitic milk and from the corresponding ATCC strains by serial centrifugation and ultracentrifugation. Characterization is performed using Western blotting, cryo-electron microscopy, flow cytometry, and nanoparticle tracking analysis (NTA). Their biological effects are evaluated using bovine polymorphonuclear cells (PMNs) as a proxy for somatic cells present during mastitis. Three different concentrations of each BEV protein preparation were tested. Cell viability and proliferation are assessed using the WST-1 assay to determine cytotoxic effects, while inflammatory activation is evaluated by measuring inflammasome-associated caspase-1 activity. Preliminary observations suggest that wild isolates may induce higher levels of cell death and a stronger upregulation of pro-inflammatory mediators compared to ATCC reference strains. E. coli treatments appear to be associated with a more pronounced acute inflammatory response, whereas S. aureus may exhibit a comparatively moderate but potentially sustained effect. BEVs derived from wild isolates are expected to trigger measurable inflammatory signaling, supporting their possible contribution to host–pathogen interactions even in the absence of viable bacteria. These findings highlight potential differences between laboratory reference strains and field isolates in their capacity to modulate inflammatory responses. Understanding these variations may improve knowledge of mastitis pathophysiology and support the development of more accurate in vitro models and diagnostic biomarkers for dairy health management.

Social Intermittent Suckling: Easing Weaning for Piglets and Sows

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This study compared conventional continuous suckling (CW; n = 17 sows) with social intermittent suckling (SIS; n = 18 sows) to evaluate sow energy balance and piglet performance before weaning (24 d) and during the post-weaning period. In the SIS treatment, sows were progressively separated from their litters for 4 to 10 h/day during the last 11 days pre-weaning, while piglets were co-mingled and had access to creep feed. Post-weaning performance was evaluated in a split-plot design using electronic feeding stations, with piglets housed in 12 pens of 12 animals each (n = 144). Despite similar feed intake (~5.5 kg/day), SIS sows experienced a less negative net energy balance compared with CW sows (–20 vs. –30 MJ/day; $P < 0.01$), as estimated using Noblet and Dourmad equations adjusted for creep intake. This was associated with lower milk energy output (48.4 vs. 56.2 MJ/day) and reduced milk protein yield (493 vs. 538 g/day; $P < 0.05$). Consequently, SIS sows mobilized less body protein (70 vs. 147 g/day; $P < 0.01$) and fat (341 vs. 520 g/day; $P = 0.01$), and tended to lose less backfat (3.6 vs. 5.1 mm; $P = 0.081$). Before weaning, SIS piglets consumed more creep feed than CW piglets (52.8 vs. 24.9 g/pig/day; $P < 0.001$), with peak intakes reaching ~105 g/pig/day. Creep feed contributed ~13% of energy intake and ~14% of growth in SIS piglets, compared with ~5–6% in CW piglets ($P < 0.001$). This partial substitution of milk resulted in lower pre-weaning average daily gain (222 vs. 261 g/day; $P < 0.01$) and lighter weaning weight (6.3 vs. 6.8 kg; $P < 0.01$). During the early post-weaning period, SIS piglets showed markedly higher feed intake and average daily gain (+66–67%; $P < 0.001$). These differences persisted throughout the nursery phase, resulting in 2.2 kg higher body weight at day 34 post-weaning (19.6 vs. 17.4 kg; $P = 0.001$). In conclusion, SIS reduced sow metabolic load during late lactation and promoted early solid feed intake by piglets, resulting in accelerated post-weaning growth despite a modest reduction in pre-weaning performance. SIS appears to be a functional management strategy to support gradual weaning while preserving sow condition.

The future perspectives of increasing within-litter body weight uniformity in pigs

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Increasing within-litter body weight uniformity remains a major challenge in modern pig production. In a 4 year research project, we demonstrated that lower uniformity of body weight is associated with more lightweight piglets, reduced growth performance and increased mortality. Factors influencing weight uniformity were investigated across the entire lifespan of pigs and strategies to reduce variation throughout the production cycle were evaluated. Uniformity metrics were strongly correlated, indicating that simpler measures such as within-litter weight range or the proportion of lightweight piglets can reliably replace more labour-intensive metrics that require weighing all piglets individually, like coefficient of variation or standard deviation. However, we think that future progress will depend strongly on improved phenotyping. Automated technologies, such as computer vision and precision livestock systems, may enable continuous and objective monitoring of body weight, feed intake, farrowing processes and growth stability, reducing labour demands and advance our understanding of within-litter body weight uniformity. These innovations are expected to make large-scale monitoring of uniformity traits more feasible. In a first study, we showed that uniformity is dynamic, with major shifts around weaning. Weak correlations were found between birth weight uniformity and post-weaning uniformity indicating that these are two distinct traits: one before weaning, closely linked to pre-weaning survival and one after weaning, relevant during the finishing phase and at slaughter. Larger litters, more mummified and stillborn piglets, higher sow parity and larger chest circumference were associated to an increased body weight variation, whereas a higher number of functional teats was linked to higher uniformity. Individual growth performance depended on birth interval, sex ratio, sow body condition loss, litter size and weaning age, emphasizing the need to balance litter size with the sow's capacity. As maternal features play a major role, breeding goals may need to shift from maximizing litter size to optimizing the number of uniformly growing, robust piglets relative to the capacity of the sow. Second, we tested the effect of feeding interventions around weaning, which showed only short-term effects on body weight uniformity. However, literature suggests that the gut microbiome may influence early-life growth trajectories and piglet robustness. New approaches may be more succesful. In a third phase, we conducted an intervention trial to evaluate a genetic strategy to reduce variation. Estimated breeding values for uniformity and survival of terminal Piétrain sires showed only weak correlations with within-litter uniformity in their crossbred offspring, indicating limited predictive value for this trait. Nonetheless, meaningful associations were observed with growth stability, suggesting that uniformity and resilience traits could contribute to more refined breeding goals. Overall, the results demonstrate that within-litter uniformity is shaped by a complex interplay of maternal, genetic and management factors. Future pig production should shift its focus from increasing litter sizes to increasing piglet quality, which will eventually benefit the animals, the farmers and the pig industry as a whole.

Dietary combination of β-galactomannans, plant extracts and monoglycerides improves survival and performance in post-weaning piglets

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During the post-weaning period, piglets are highly susceptible to enteric disorders which negatively impact their health, welfare and overall productivity. Hydrolysable β-galactomannans (HβG) may compete with pathogens for epithelial adhesion and exert prebiotic effects and can modulate intestinal morphology and cytokine expression. In combination with phytogenic extracts (PHY) and short- and medium-chain fatty acid (FA) monoglycerides, these compounds may enhance resilience during this stage. The hypothesis of this study was that the inclusion of 3,000 ppm of a complementary feed containing HβG, selected PHY, and short- and medium-chain FA monoglycerides, would improve survival and growth performance of weaned piglets reared under intensive farming conditions. A total of 252 piglets (Large White × Landrace females × Pietrain males; 24 d of age; 6.3 ± 1.44 kg) were allocated to 21 pens (12 piglets/pen), in two rooms, in a randomized design with two treatments: control diet and control + 3,000 ppm of a dietary combination of HβG, phenylpropanoids from PHY, and short- and medium-chain FA monoglycerides (EG; ENAGUT®). The trial lasted 42 d (pre-starter: d0–14; starter: d15–42). Growth performance, mortality, health status and faecal consistency (Hu et al., 2012) were recorded periodically. Pen was the experimental unit. Data were analysed using a mixed-effects model with repeated measures (SAS 9.4), including treatment, period and their interaction as fixed effects, and pen and room as random effects. Results are expressed as mean ± SEM and significance was declared at $P < 0.05$ and trends at $P \leq 0.10$. During the starter phase, EG reduced mortality to 0.00% compared with 6.82% in control group ($P < 0.01$). Overall, mortality over 42 d was also lower in supplemented piglets (2.37 vs. 7.50%; $P < 0.05$). No significant differences were detected in feed intake or faecal consistency. A trend of increase in the average daily gain vs. control was observed during the starter phase (0.40±0,08 vs. 0.38±0,09 kg/d; $P = 0.09$). Over the whole study, supplemented piglets showed numerical improvements in final body weight (19.30±4,17 kg vs. 18.59±4,32 kg; +3.7%), average daily gain (0.31±0,07 kg/d vs. 0.29±0,07 kg/d; +6.5%), and feed conversion ratio (1.48±0,15 vs. 1.53±0,34; −3.4%), although these were not statistically significant ($P > 0.05$). The absence of differences in faecal consistency suggests that improved survival was not primarily associated with reduced diarrhoeal expression but may reflect systemic effects on host resilience. Nutritional modulation of immune function is recognized as a key determinant of robustness during weaning. The survival advantage observed with EG may therefore involve enhanced immune competence, improved inflammatory balance, and strengthened mucosal defence mechanisms, as well as control of microbial load and dysbiosis. From a production perspective, reductions in post-weaning mortality exert a leveraged effect on whole-cycle profitability by increasing the number of pigs entering the grower–finisher phase and optimizing facility utilization. Thus, beyond performance metrics alone, enhanced early-life survival represents a relevant driver of productive efficiency and economic sustainability. In conclusion, supplementation with 3,000 ppm EG improved significantly survival and tended to enhance growth performance in post-weaning piglets reared under intensive conditions.

Effect of supplementing gestating and lactating sow with a Citrus-based sensory feed additive on sow reproduction and piglet performance

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Essential oils and plant extracts have shown beneficial effects on stress management in swine. The aim of this trial was to assess the effects of supplementing gestating and lactating sow with a sensory feed additive, based on Citrus sinensis essential oil, on sows' tear staining, reproductive performance, and piglet growth. This trial was conducted in a commercial farm in France. A total of 86 sows (Hypor) were monitored across four batches: two control batches (n=37) and two treated batches (n=48). Parity distribution was balanced between groups to avoid experimental bias. Both groups received standard gestation, peripartum, and lactation diets according to their physiological stage. The treated group received a supplementation with a citrus-based sensorial feed additive (Laboratoires Phodé) at 250 g/tonne of feed. Litter body weight was measured at day 7 and day 28, from this, litter growth was calculated. Sow body condition was evaluated by measuring backfat thickness using a backfat meter (Renco, Schippers, France) at day 7 and day 28. Backfat differences was calculated. Tear staining was scored using a 5 points scale (0 = no staining to 4 = severe staining), as a marker of stress status. All data were analysed by a Student's t-test using SAS software, except the tear staining scores, which were analysed by a Khi square test using SAS software. The average number of piglets at day 7 were similar between treatment groups. Litter weight at weaning (day 28) was higher by 3.7 kg in the treated group compared to the control group (65.8 vs 69.4 kg; $p = 0.1$). Backfat loss during lactation was reduced by 2 mm in the treated group compared to the control group (-4.1 vs -2 mm; $p=0.05$). Tear staining scores differed significantly between groups ($p<0.001$). There was a higher proportion of animals classified in score 1 in the treated group compared to the control group (9.6 vs 31 %). The control group presented a greater percentage of sows in score 3 compared to the treated group (40 vs 20.7 %). There were no sows presenting score 4 in the treated group and 15.4 % in the control group. Supplementing sows with specific sensory feed additive improves the psychosocial state of sows during lactation, limits the loss of body condition in sows during lactation, and improves litter weight at weaning.

Effects of different combinations of dietary zinc and copper sources on their homeostasis, mitochondrial function, ATP concentrations and antioxidant response in different tissues in post-weaned piglets

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The restrictions on the use of zinc oxide (ZnO) during the post-weaning period in the European Union have intensified the discussions to reduce pharmacological Zn levels worldwide. In Canada, total dietary Zn will be limited at 300 ppm although it is still not clear if such levels are sufficient to meet immediate post-weaning requirements and sustaining robustness. This study evaluated the effects of inorganic (I) and organic (O) Zn (ZnO or Zn proteinate) and Cu (CuSO4 or Cu-amino acid complex) sources on their homeostasis and metabolic health parameters in pigs. At weaning (d21), 90 piglets were randomly assigned to four diets combining Zn and Cu sources (IZnICu, OZnOCu, IZnOCu, OZnICu) at 300 and 130 ppm, respectively. Samples were collected at d21, 28, and 35 to evaluate Zn and Cu concentrations and antioxidant response in plasma, jejunum mucosa, liver, and mRNA expression of selected genes, mitochondrial Zn and Cu concentrations, cytochrome c oxidase activity, and ATP concentrations in jejunum mucosa and liver. Statistical analysis compared each treatment vs pre-treatment levels (d21) in addition to a 2×2 factorial contrast between the different combinations of sources at d28 and 35. Compared to d21, plasma and liver Zn concentrations were lower in all treatments at d28 and 35 ($P \leq 0.08$), except plasma Zn in IZn groups at d35 that was not different from d21 ($P \geq 0.45$). Plasma and hepatic Cu concentrations were not different from d21 ($P \geq 0.20$) whereas plasma Cu did not differ among treatments ($P \geq 0.18$) but hepatic Cu tended to be higher in IZn piglets than OZn at d28 ($P = 0.07$). Jejunal ZIP4 mucosa mRNA expression at d28 was enhanced by IZn only when combined with ICu (interaction; $P = 0.07$). In jejunum mucosa ($P \leq 0.09$) and liver ($P \leq 0.05$), IZn increased MT3 expression at d28 and 35. The expression of ZIP5, ZnT1, CTR1, ATOX1, ATP7A (jejunum only), and ATP7B (jejunum and liver) were not affected by treatments ($P \geq 0.12$). At d28, OZn increased jejunal mitochondrial Zn in OCu piglets but not ICu (interaction; $P = 0.06$) whereas hepatic mitochondrial Zn was higher in IZn than OZn groups ($P = 0.05$). Hepatic cytochrome c oxidase activity increased only in OZnOCu piglets (interaction; $P = 0.05$) and hepatic ATP concentrations tended to be greater in OZn groups ($P = 0.10$). No treatment effect was detected on plasma and jejunal SOD and GPX activities or carbonyl protein concentrations ($P \geq 0.12$). Liver SOD activity was higher for OZn piglets than IZn (d35, $P = 0.01$; trend at d28, $P = 0.10$), and higher for OCu piglets than ICu at d28 ($P=0.05$). Carbonyl protein concentrations at d28 tended to be lower for OCu than ICu groups ($P=0.10$). In conclusion, regardless of source, 300 ppm Zn was not sufficient to maintain body Zn status during the first post-weaning week but, in combination with 130 ppm Cu, avoided the commonly observed drop in Cu status after weaning. Piglets fed IZn showed increased MT3 expression and hepatic mitochondrial Zn accumulation whereas OZn demonstrated superior functional hepatic outcomes related to ATP synthesis and antioxidant response, suggesting better functional accessibility of the organic source.

The use of blood biomarkers to evaluate the impact of acute heat stress in piglets and growing finishing pigs

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Heat stress (HS) disrupts metabolic, physiological, and immune functions in a systemic manner, ultimately compromising growth and overall performance of animals. Pigs are particularly vulnerable to HS, and their capacity to dissipate excess heat decreases as they grow, making older animals more susceptible. A better understanding of the physiological responses to HS using minimally invasive approaches is essential to predict, prevent, and monitor the effectiveness of mitigation strategies. This study assessed acute HS induced changes in piglets and growing finishing (GF) pigs using blood biomarkers. A total of 96 pigs (21d old; 6.7-88.7kg BW over 112 days) were housed at thermoneutral temperatures across production phases. Pigs were offered commercial diets at NRC levels. Animals were exposed to two acute HS challenges: piglets on days 26-28 (6 h at 36-38 °C; 18 h at 30-32 °C), and GF pigs on days 54-56 (6 h at 30-32 °C; 18 h at 28 °C). One pig per pen was sampled on d 25, 29, 53 and 57. Blood biomarkers were analyzed for hematology, oxidative stress biomarkers via commercial assay kits, point of care devices iSTAT CG8+ cartridge, and VetScan Comprehensive diagnostic profile. Data were analyzed in JMP Pro 18 using a repeated-measures mixed model, with day as the repeated factor, pig as the subject, and an unstructured covariance matrix. Multiple comparisons were conducted using Students t-tests, with significance defined as $P<0.05$. Total protein, monocytes (count and %), and LPS-binding protein (ug/mL) were higher after HS in both phases, whereas red blood cells (10^{12}/L) hematocrit (%) and alkaline phosphatase (U/L) were lower after HS ($P<0.05$). Additionally, neutrophils (count and %), red cell distribution width (fL and %) were lower in piglets after HS but higher in GF pigs after HS. Malondialdehyde (nmol/mL) was higher in piglets after HS but lower in GF after HS ($P<0.05$). In piglets, superoxide dismutase (U/mL) and hemoglobin (g/L) were lower after HS, whereas total antioxidant capacity (nmole/uleq), potassium (mmol/L), alkaline aminotransferase (U/L), mean corpuscular hemoglobin (g/L and pg) and mean corpuscular volume (fL) were higher after HS ($P<0.05$). In GF pigs, blood urea nitrogen (mg/dL) and glucose (mg/dL) were lower after HS, whereas eosinophils and lymphocytes (both count and %), plateletcrit (%), and platelet (10^9/L) were higher after HS ($P<0.05$). Biomarkers impacted due to HS in both growth phases could provide insights into metabolic and biological impacts of temperature on health and performance capabilities. These differences over time, especially those differing between piglet and GF phases, can demonstrate age-dependent metabolic and physiological responses to heat exposure. Changes during both HS periods may also indicate increased water consumption resulting in hemodilution. Fluctuations in the concentrations of blood hematological parameters due to HS suggest impacts on the immune and inflammatory responses, as well as oxidative stress (OS) markers, which appeared to be more impacted in the piglet phase, indicating their ability to deal with redox imbalance may be impaired during this phase. In addition, biomarkers related to metabolic parameters like BUN and glucose can provide insights into HS impacting metabolism and nutrient utilization, and these being observed in the GF phase may suggest that older animals have impaired feed intake due to HS. In conclusion, blood biomarkers provide a valuable tool to monitor physiological alterations in piglets and GF pigs subjected to short-term HS, supporting the development and validation of heat-mitigation strategies.

Participatory identification of feasible pig welfare innovations to support resilient farming systems under evolving EU legislation

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The European animal welfare regulation is currently under review to improve housing and management of pigs. However, resilience of pig production systems depends on identifying welfare improvements that are not only scientifically sound but also practically and economically feasible under commercial conditions. Structured, multi-country evidence of such implementable practices remains limited. The WelFarmers project, funded through Horizon Europe, aimed to identify and evaluate pig welfare practices that enhance system resilience while remaining feasible across diverse European production contexts. WelFarmers includes 8 regional networks established in Denmark, Finland, France, Ireland, Italy, Portugal, Romania and Spain representing more than 50% of the pig herd across the EU. A bottom-up, multi-actor approach was applied, engaging farmers, advisors, veterinarians and researchers. Two rounds of good practice collection were conducted across four priority themes: loose housing of lactating sows; production of pigs with undocked tails; pain mitigation in castration / production of entire males; and space allowance & flooring for growers and finishers. A total of 200 submitted practices were evaluated using a structured multi-criteria scoring system (0-5 scale) assessing technical quality, impact, and probability of successful implementation across welfare, economic and environmental dimensions. Practices that achieved consistently high scores across the three evaluation domains were prioritised for final selection through stakeholder voting within the networks. Across themes, high-ranking practices demonstrated that welfare-oriented management and housing adaptations can be integrated into existing commercial systems without compromising operational feasibility. Strong farmer engagement throughout the evaluation process reinforced the practical relevance and transferability of the selected innovations. An environmental and economic assessment of the winning practices is being conducted to further evaluate cross-country applicability and sustainability implications. The selected practices are also being documented and presented through technical summaries, visual media and virtual farm demonstrations to facilitate knowledge transfer and uptake across the European pig sector. These results highlight the value of participatory networks in bridging the gap between legislative ambition and farm-level implementation. The identified practices provide evidence-based, field-validated examples to support resilient pig production systems capable of adapting to evolving societal and regulatory expectations.

Updated Drinking Water Requirements and Key Influencing Factors in Cattle, Pig and Poultry Production

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Drinking water supply is a critical factor for maintaining optimal performance in farm animals. In the context of climate change, water resources for animal husbandry are becoming increasingly limited, with prolonged dry periods intensifying scarcity. At the same time, ongoing genetic improvements in livestock have increased animals' performance and maintenance requirements, creating a growing need to update reference values for drinking water consumption. This study quantified drinking water requirements in three major livestock sectors, cattle, pigs, and poultry, and identified key factors influencing water consumption. Water meters were installed on 7 dairy farms, 4 beef farms, 7 pig farms and 6 poultry farms, and daily water consumption was monitored for one full year. Where possible, different animal categories (e.g. piglets, fattening pigs, lactating sows) were measured separately. The dataset comprised more than 50,000 daily observations for the three sectors, accompanied with detailed metadata including genetics, diet composition, climate parameters, (re)production parameters and drinking system characteristics. Updated drinking water consumption values were established and influencing factors were identified for each livestock sector. For beef cattle, dairy cattle, and pigs, mean water consumption values for most animal categories were higher than the reference values currently used in Flemisch environmental permits. The temperature–humidity index showed a highly significant effect on water consumption across all sectors, especially in high-performing animals such as lactating and gestating cows and sows. In dairy cattle, water consumption increased significantly with increasing milk yield ($p < 0.001$), feed intake ($p < 0.001$), and dry matter content of the diet ($p < 0.001$). In pigs, water consumption increased significantly with age in both piglets ($p < 0.001$) and fattening pigs ($p < 0.001$). Furthermore, farrowing sows showed a mean increase of 0.976 L ($p < 0.001$) per present day in the farrowing unit. In poultry, water consumption also increased significantly with age ($p < 0.001$) in laying hens and broilers, and genetic lines showed distinct differences in water consumption at equal ages. Overall, drinking water requirements were consistently higher than historical reference values used in Belgium, and water consumption was shown to be influenced by multiple biological and environmental-related factors. These updated values and insights provide essential input for more accurate water requirement estimates under changing climatic conditions.

Natural Behaviour and Activity Pattern of Pigs: A comparative study of breeds and environments

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Understanding pigs' natural behaviour is essential for improving housing and management practices that promote their behavioural expression and ensure their needs are met. This study therefore aims to improve the understanding of pig behaviour by examining daily activity patterns, natural behaviours, and the influence of breed and environment. The research addressed the following questions: How do pigs allocate their time across daily activities? Are there behavioural or activity differences between breeds? Do rooting, resting, play behaviour, and social interactions differ between pigs housed in stables and those kept in field conditions? Furthermore, it was hypothesized that there would be no significant differences in daily activity patterns between breeds, while pigs in field conditions would show more rooting and play, less resting, and fewer negative social interactions compared to stable-housed pigs. Two behavioural experiments using direct observation were conducted for a total of 48 days; 24 days for each experiment divided over three periods. The first experiment investigated three breeds (Angeln Saddleback, Linderöd, and Mangalitza) in a semi-natural environment. All observations were conducted between 9:10-16:10 with each observation session lasting 20 minutes. The behavioural observations were based on continuous group sampling and the ethogram covered the following behaviours: eating, drinking, foraging behaviour, locomotion, resting and sleeping, play behaviour, eliminative behaviour, social behaviour, sexual behaviour, comfort behaviour and others. The second experiment compared slaughter pigs housed on the same organic farm in organic stables with those kept in field accommodations. All observations were conducted between 9:15-16:30 and one observation round took 31 min. The ethogram covered the following behaviours: rooting, resting, social interactions and play behaviour, with scan sampling used for rooting, resting and social interactions and play behaviour recorded with event sampling. Data were analyzed in R. Differences between breeds were analyzed using a Kruskal–Wallis test for all behaviours. When significant, pairwise Mann–Whitney U tests were conducted to identify specific breed differences. A Bonferroni correction was applied to control for Type I error. Differences between housing systems were analyzed via Mann– Whitney U tests carried out for each behaviour defined in the ethogram. Results showed that pigs in semi-natural environment devoted most of their active time to foraging (20–40%) and eating (17–20%), while resting accounted for 12–44% depending on breed. Significant breed differences ($p < 0.05$) were found between Linderöd and the two other breeds in the duration of the behaviour categories: eating, resting, drinking, elimination and others, with Linderöd being the most active breed. In the study concerning slaughter pigs, pigs with field access rooted and played significantly more ($p < 0.05$) and rested less during daytime activity than pigs in stables, supporting the hypothesis that environment strongly shapes behavioural expression. Overall, the findings highlight that pigs are highly active animals with a strong need to forage. This holds despite the fact that breed may influence the duration of certain behaviours. Housing environment had a clear impact, underlining the importance of providing conditions that enable pigs to express their natural behaviour and thereby improve welfare.

Changes in body dimensions of Irish sows over 20 years

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The phasing out of traditional farrowing crates is currently being discussed by the EU commission. This presents an opportunity for adoption of well-designed alternative housing which requires an understanding of the needs of the animals, knowledge of sow body dimensions and changes in litter size to ensure the provision of appropriate space to perform natural behaviours. The aim of this study was to measure the body dimensions of sows on Irish commercial farms and evaluate changes since similar work was conducted in 2005. The body dimensions of 490 sows across all parities from across four Irish commercial farms were measured. Sows were measured from snout to the tail base (length), from the floor to back (height), from udder to back (depth), and across the front shoulders (width) using a measuring tape, calipers, and spirit level. Sows were in farrowing crates 1-2 days prior to farrowing. Data were analysed across parity and between farms to assess trends in size. The data were compared to sow body dimension data collected in 2005. Among all sows sampled, body dimensions ranged from 154cm to 205cm (length), 75cm to 105cm (height), 52cm to 78cm (depth), and 28cm to 49cm (width). Across parity, body dimensions followed the expected pattern. As the sows got older there was a significantly increase ($p < 0.05$) up to parity 5, but an unexpected small decrease in size was present in all dimensions at parity 6. In contrast, the earlier findings showed Irish sows continuing to grow in height, depth, and width up to parity 8. Between farms, variation was observed in mature sow (parity 5+) size in dimensions of height ($p < 0.001$) and width ($p <0.01$). For each dimension, at least one farm had significantly larger mature sows compared to the 2005 values ($p < 0.05$). The observed decline at parity 6 may reflect culling practices, where larger sows are preferentially removed or more susceptible to lesions/locomotion issues, reducing the representation of large sows in late parities. Variation between individual farms and 2005 sows may be due to differences in management, genetics, or culling selection strategies influencing sow body size outcomes. Sow body dimensions have increased since 2005, consideration of these changes is an important aspect of designing appropriate housing for the sow's needs.

Botanical supplementation: An effective Approach to manage tail biting in pigs farm

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Building resilient livestock systems may require new tools against welfare issue. Regarding the tail biting behavior, a multi-factorial issue, most of the currently identified solution requires medium- to long-term measures, like reducing density or working on air flow. In this context, liquid complementary feed can offer a short-term solution to preserve the system health before working on other measures. By their documented calming effect on animal's behavior, Melissa officinalis and magnesium are relevant to enter in such a solution. This study assessed the impact of a natural solution containing Melissa officinalis and magnesium supplementation used in reaction to tail biting and its effects on pigs' behavior under commercial conditions. After weaning at 28 days, piglets from a gilt-breeding farm were randomly allocated to either a supplemented (S, n=103) or a control (C, n=102) group in identical environments, under commercial conditions. To evaluate tail biting evolution inside of each group, and its consequences, all pig tails were graded once a week from week 7 to week 21. Supplementation started in reaction to fresh blood observation, the supplement (Care Zen) was added to the drinking water (1 L/1000 L) for a 5-day period. The choice of a reactive approach was done to get close to real farm management practices. Tail grades between groups were compared using a Chi-square test with pig as a statistical unit. For behavioral analysis, in both rooms, one pen was video-recorded at three different periods for 3 consecutive days. The videos were analyzed using the scan sampling method to assess tail biting, resting, exploring, and feeding behaviors. Each scanned hour was defined as an observation period (OP), and was the statistical unit for behavioral analysis. In total, 97 OP were analyzed. Principal component analysis and hierarchical ascending classification were used to objectively analyze behavioral data and create OP clusters defined by the prevalence of pig behaviors within each. Number of tail or ear biting events in each group were analyzed through a Fisher test, with OP as statistical unit. All assessments were carried out by a blinded evaluator. In total, two supplementation periods took place. On camera recordings, tail and ear biting observations per day were significantly lower for S (Fisher, p-value <0.05), with 0.44 +/-1 events than in group C with 13.74+/- 10 (mean ± SD) events per day representing a 96.8% reduction in biting events. From video observations, three OP groups were identified based on behavior: Low, Medium, and High activity hours. The C group contained all (14/14) High activity OP with more aggressive behaviors (Dunn, p-value < 0.05), while the S group included more (79%, 19/24 in S) Low activity OP. Medium activity OP appeared equally (10/22 in S) in both groups and are mainly linked to feeding times or non-aggressive playful activities. These results show that a reactive supplementation with a standardized botanical product may contribute to reducing tail biting and its severity and induce a calmer atmosphere on farms. We thank Alexis Nalovic (Epidalis, Vitré, France) for identifying the study farm and for providing technical support throughout the study, allowing the evaluator to remain blinded.

Ex vivo antimicrobial assay determines in vivo antibacterial activity of glycerides of lauric acid against S. suis

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Antibiotic resistance is an increasing threat, making alternative strategies essential for controlling pathogens such as Streptococcus suis in pigs. We developed an ex vivo antimicrobial assay using digestive fluids from animals supplemented with glycerides of lauric acid (GC12). We hypothesized that digestive fluids from piglets receiving GC12 would exhibit higher antibacterial activity against S. suis compared to digestive fluids from non-supplemented piglets. Twelve weaned piglets of 24 days of age were equally divided into two groups. A control group received a commercial diet adequate in all nutrients, and the treatment group received the same commercial diet supplemented with GC12 (FRA® C12 Dry) at 4 and 3 kg/ton of feed from 0 to 11 days and from 12 to 50 days, respectively. At day 50, all piglets were euthanized by an intracardial overdose of sodium pentobarbital 20% until cardiac arrest, after intramuscular sedation with a mixture of xylazine, tiletamine and zolazepam (XylM 2% + Zoletil 100) at a dose of 0.22 mL/kg bodyweight. Digesta samples were then collected in Falcon 50 ml tubes from the ileum, distal colon, and rectum. Digestive fluids were extracted by centrifugation and filtration with 0.22µm filters and then subjected to MIC analysis to determine antibacterial activity against S. suis CRBG BB-3 serotype 7 in a 1:1 dilution with the bacterial culture. Fisher's Exact Test (one-sided) was used to analyze statistical differences of the binary outcomes (0 = growth, 1 = inhibition) from the control and treatment group. Antibacterial activity was observed in 17% of the digestive fluid samples collected from both the ileum and distal colon of the control piglets. Rectal samples showed higher activity with 40% of the samples having inhibitory effect on S. suis. In GC12-supplemented piglets, significantly higher inhibition ($P = 0.04$) was observed in the ileum as compared to the control group, with 83% of the samples showing antibacterial activity. In the distal colon, S. suis inhibition was obtained in 67% of the samples ($P = 0.12$) and 60% of the rectal samples ($P = 0.50$). Previous studies have already demonstrated in vitro antibacterial activity against S. suis, however, determining the effective dose level in vivo based on minimum inhibitory concentration (MIC) values is challenging, as multiple physiological processes including, digestion, absorption and microbial interactions, can substantially modify the bioavailability and activity of the compound. Therefore, we developed this ex vivo antimicrobial assay using digestive fluids from animals supplemented with the feed additive. Indeed, the digestive fluids from piglets receiving the recommended dose level of GC12 showed higher antibacterial activity against S. Suis compared to digestive fluids from non-supplemented piglets with the most pronounced effect observed in the ileum, a key segment of the intestine where immature immunity, high nutrient availability, and epithelial vulnerability can facilitate early bacterial translocation and systemic dissemination. Hence, our findings align with previous MIC data, proof that the in vivo dose level is effective and supports the hypothesis that GC12 supplementation can contribute to mitigation of bacterial challenges as S. suis in vivo. The lack of significant difference in distal colon and rectal samples may reflect the limited number of replicates or inherent variability in antimicrobial activity along the intestinal tract. A follow up study should further assess if rectal samples can serve as a reliable matrix for evaluating the ex vivo antimicrobial effect of feed additives.

Plasma lipid dynamics during traditional acorn-based fattening in culled Iberian sows

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The montanera fattening system, based on free-range grazing of acorns and grass in the dehesa ecosystem, is a traditional extensive production model for high-quality Iberian pig products. It offers a sustainable strategy by economically valuing culled sows within a circular economy framework. Although effects of acorn-based diets on meat quality, particularly intramuscular fat content and fatty acid profile, have been documented, the associated metabolic responses in blood lipid during this fattening phase are not yet fully characterised. Understanding these physiological changes in entire culled sows is essential to explaining the biological basis of high-quality meat production. This study aimed to evaluate plasma lipid profile dynamics during a 60-day montanera fattening period in culled Iberian sows. A total of 18 culled Iberian sows (initial body weight 162.4 ± 3.29 kg) were studied during traditional montanera. All animals were entire (non-castrated) to avoid hormonal confounding factors. Blood samples were collected at the beginning and end of the fattening period. Plasma was analysed for high-density lipoprotein cholesterol (HDL), low-density lipoprotein cholesterol (LDL), total cholesterol, triglycerides, glucose, and urea. Acorns from Quercus ilex, the predominant food source, contain high starch (50-60%) and low protein (4-6%), with lipids rich in unsaturated fatty acids, particularly oleic acid (>60%). Animals reached a final body weight of 203.8 ± 3.25 kg, with backfat thickness measured by ultrasound increasing from 3.09 ± 0.21 to 4.92 ± 0.29 cm. The montanera phase induced profound shifts in lipid metabolism. HDL cholesterol increased from 49.6 ± 10.0 to 82.4 ± 15.7 mg/dL (P=0.016), a 66% elevation. Total cholesterol increased from 112.1 ± 22.5 to 159.7 ± 31.6 mg/dL (P=0.017), representing a 42% rise. Most remarkably, triglycerides increased by 139%, from 82.2 ± 35.5 to 196.7 ± 70.9 mg/dL (P<0.001). Glucose increased from 71.9 ± 5.9 to 90.7 ± 8.8 mg/dL (P=0.001). In comparison, urea substantially decreased from 31.5 ± 6.0 to 12.8 ± 6.0 mg/dL (P<0.001), a 59% reduction suggesting efficient utilisation of acorn-derived lipids and carbohydrates as primary energy sources, with reduced reliance on protein catabolism. These results demonstrate that traditional acorn-based fattening promotes major lipid metabolism adaptations in entire culled sows, characterised by substantial elevations in circulating lipoproteins and triglycerides. The marked increase in HDL cholesterol alongside a substantial elevation in triglyceride reflects a metabolic response to an acorn-based diet rich in unsaturated fatty acids. Concurrent backfat thickness gains and substantial urea reduction indicate efficient nutrient partitioning towards lipid deposition rather than protein degradation. These physiological changes provide the foundation for understanding the characteristic intramuscular fat infiltration and favourable fatty acid composition that define montanera-finished Iberian pig products. This work contributes to characterising plasma lipid dynamics during montanera fattening in culled Iberian sows, providing insights into the metabolic foundation of this traditional production system and supporting the valorisation of culled Iberian sows.

Implementing new legal requirements for sow housing: factors influencing functional use of an outdoor area in the mating area

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In response to the European Commission's 2021 initiative to phase out permanent confinement, legislative reforms in Germany will require group housing with short-term fixation and at least 5 m2 per sow in the mating area by 2029. This transition presents structural and management challenges, particularly during the critical post-weaning phase characterised by social reorganisation and oestrus activity. Integrating an outdoor area offers a practical approach to increase functional space and enhance welfare. However, evidence-based design requires a detailed understanding of utilisation patterns and influencing factors. Therefore, the use of an outdoor area was investigated under commercial farm conditions. A total of 85 sows were observed on 14 selected observation days between March and August 2023, with a focus on the first four days after weaning (day 0–3). Outdoor presence and resting behaviour were recorded at 10-minute intervals using video analysis. Environmental parameters, a heat stress index, and group composition were analysed using generalised linear mixed models. Outdoor area use showed a clear diurnal pattern ($P < 0.001$), with peaks shortly after the morning feeding and in the afternoon. On average, 32.7% ± 14.0% of sows were outdoors. During periods of low overall outdoor presence, particularly at night, most sows present were resting, whereas during peak activity in the morning, resting decreased sharply, indicating a functional shift from resting to activity and social interaction. Social dynamics influenced early post-weaning use ($P < 0.05$). Outdoor presence was highest on day 0, while resting was minimal, reflecting exploratory behaviour and hierarchy establishment. In subsequent days, presence decreased and resting increased, indicating progressive social stabilisation, with a renewed increase in outdoor presence on day 3, linked to oestrus activity. Greater parity variation within groups reduced outdoor presence ($P < 0.001$), suggesting that social heterogeneity may limit equal access. Environmental factors significantly modulated use patterns. Precipitation consistently reduced both presence ($P < 0.001$) and resting behaviour ($P < 0.05$), while solar radiation promoted outdoor presence ($P < 0.001$). Higher thermal load decreased both outdoor presence and resting behaviour ($P < 0.001$). Overall, the outdoor area was used dynamically as a social, active, and resting space. Effective design should consider temporal functional shifts, conflict-reducing spatial structuring, and protection against adverse weather. These design considerations facilitate the practical implementation of the 5 m2 space requirement in group-housed mating systems. This work is financially funded by the Federal Ministry of Agriculture, Food and Regional Identity (BMLEH) based on a decision of the Parliament of the Federal Republic of Germany, granted by the Federal Office for Agriculture and Food (BLE; grant number: 28N305603).

Behaviour of fattening pigs in structured pens with outdoor access

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Due to climate change and the increasing emphasis on animal welfare, German pig housing systems are undergoing substantial transformation. Structured pen designs are gaining popularity because they can better accommodate pigs' behavioral and physiological needs by providing distinct functional areas. This study investigated the use of a structured pen with outdoor area at different times of day. The study was conducted on a commercial farm in Germany from March to September 2025. A total of 168 pigs (Topigs x Duroc) kept in two structured pens were examined over the course of two batches. Each pen was divided into functional areas, including two separate lying areas, a slatted floor area and an outdoor run with straw bedding. Temperature and humidity were continuously monitored using data loggers. Video recordings were analyzed using a scan sampling approach: The use of the different functional areas was assessed for a full day once per week. For this purpose, the number of pigs located in the outdoor run, lying area and on the slatted floor was counted on day (8AM, 10AM, 12AM, 14 PM, 16PM) and at night (8PM, 10PM, 2AM, 4AM). Additionally, the body positions prone, lateral, standing, and sitting were recorded. The data were descriptively described. The mean number of pigs differed between functional areas and between day and night periods. During the night, pigs were mainly located in the outdoor area (mean and standard deviation: 16.1 ± 33.1 pigs) and in the lying area (10.3 ± 19.8 pigs), whereas fewer animals were observed in the slatted floor area (3.9 ± 7.7 pigs). During the day, most pigs were found outside (23.3 ± 25.8 pigs). Comparable numbers of pigs were present in the indoor areas, with 9.0 ± 10.1 pigs in the lying area and 9.0 ± 8.6 pigs in the slatted floor area. The mean number of pigs exhibiting different body postures varied between day and night periods. Outside, lateral lying was the predominant posture during both time periods, with 56.8 ± 29.5 pigs lying laterally during daytime (06:00–16:00) and 60.4 ± 42.1 pigs during nighttime (18:00–04:00). In contrast, standing outside occurred significantly more frequently during the day (17.4 ± 8.5 pigs) than during the night (1.5 ± 1.9 pigs). The outdoor run was well accepted by pigs throughout the entire day in the analysed warm period, indicating successful integration into the housing system independent of time of day. Pigs used the outdoor area for both resting and active behaviors, demonstrating its function as a multifunctional space rather than solely an activity zone. Frequent lateral lying suggests that animals perceived the outdoor environment as comfortable and suitable for undisturbed rest, while increased standing behavior during daytime reflects active exploration and interaction with the provided straw as environmental enrichment. Continuous use of the outdoor run highlights its relevance for supporting natural behavioral expression and promoting animal welfare. Overall, structured pens improve pig welfare by offering a range of functional areas allowing behavioral flexibility. This research is part of the ZERN (Future Nutrition Lower Saxony) network and was funded by the Lower Saxony Ministry for Science and Culture (MWK) and the Volkswagen Foundation within the programme zukunft.niedersachsen.

Effects of different nesting materials on crushing risk and sow posture changes in a temporarily confined farrowing system

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The provision of nesting material is regarded as an important measure to support pre-parturient behavior in sows. However, under commercial conditions its practical implementation remains limited, partly due to practical concerns such as the potential negative effects on manure systems and additional labor requirements. The present study evaluated the impact of different organic nesting materials on crushing risk and sow activity in conventional farrowing systems with short-term crating. At the same time, the aim was to generate practice-oriented evidence in line with current animal welfare regulations, to address existing practical reservations. In this study, a total of 263 farrowings from 141 sows were included. Each sow was randomly assigned to one of three treatments: grass hay, long-stemmed straw, or a jute bag serving as minimal enrichment control. Nesting material removal from the racks was recorded daily from day 112 of gestation until farrowing. Crushing losses were documented during the first three days postpartum. In addition, sow activity during the first 72 h after farrowing was quantified using automated video-based analysis, with posture changes (transitions between standing and lying) serving as an objective indicator. Although considerable inter-individual variation occurred, no significant differences in pre-partum material usage during the two days prior to farrowing were detected between hay and straw (means: 417 g vs. 354 g). Crushing losses within the first three days after birth were lowest in the straw group (3.51 %), followed by hay (4.68 %) and the jute bag group (6.52 %). Statistical analysis showed 46 % lower odds of crushing in the straw group compared to jute bag control, whereas the hay group showed no significant differences to the jute bag treatment. Sows provided with straw exhibited significantly fewer posture changes (median 128) than sows from the jute bag control (median 160), corresponding to a reduction of approximately 28 %. A moderate positive correlation ($\rho = 0.40$) was observed between posture change frequency and crushing losses during the first three days postpartum. The results demonstrate that not only the provision but also the functional properties of nesting material are relevant. Long-stemmed straw reduced sow activity during the critical early postpartum phase and significantly decreased crushing risk. Furthermore, the small quantities of used material in this study are unlikely to interfere with manure systems. Thus, even in conventional systems with partially slatted floors and temporary crating, structured nesting material represents a practical and feasible management strategy to improve sow and piglet welfare. This work is funded by the German Federal Ministry of Agriculture, Food and Regional Identity (BMLEH) based on a decision of the Parliament of the Federal Republic of Germany, granted by the Federal Office for Agriculture and Food (BLE). Funding code: 28N305603

Does the temperature-humidity index affect the growth rate of weaner pigs?

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Measuring pigs´ individual body weight via automatic 3D-image-processing is valuable for analysing the weight distribution within a pen or batch and for assessing weight gain without the stressful and time-consuming procedure of manual individual weighing. This study investigated whether any impact of the temperature-humidity index (THI) is reflected in automatically generated data on weekly weight gains of individual weaner pigs. Seven batches of weaner pigs (n=1,446) with RFID-ear tags housed in one compartment with six pens, equipped with climate sensors (DOL 114, dol-sensors A/S, Aarhus, Denmark), were included in this study. Modified drinking stations with RFID-antennas and 3D-cameras (OptiTrack, Hölscher + Leuschner, Emsbüren, Germany) automatically assigned the RFID-number to the estimated optical weight (EOW) continuously. During the six weeks of rearing, the mean weekly THI (wTHI) was calculated from the recorded climate data according to De Prekel et al. (2024), and EOW were summarized weekly by using the mean weight (mEOW) for the specific period. After excluding implausible weights (<5 and >40 kg), the percentage increase between the individual weights of consecutive weeks was used to calculate relative weekly gains (rWG). A spearman correlation (ρ) between the rWG and wTHI was calculated for each rearing week (1-6). The wTHI values recorded over all batches and weeks ranged from 71.9 to 82.2, with an average of 77.1. From week 1 to 2 ($\rho = -0.058$, $p = < 0.001$, $n = 5{,}982$), 2 to 3 ($\rho = -0.146$, $p < 0.001$, $n = 5{,}964$) and 5 to 6 ($\rho = -0.119$, $p < 0.001$, $n = 5{,}778$), there were highly significant, but very weak to negligible, negative correlations between rWG and wTHI. There was no significant correlation in weeks 3 to 4 ($\rho = 0.019$, $p = 0.144$, $n = 5{,}970$), and a negligible positive influence in weeks 4 to 5 ($\rho = 0.069$, $p < 0.001$, $n = 5{,}898$). A clear influence of the THI on individual relative weekly weight gains in weaner pigs was not demonstrated based on optical weight measurements. Since multifactorial influences (e.g. genetics, feed, health status, animal welfare) can affect growth rates and heat stress-resilience as well, further research is needed to evaluate the effect of elevated THI-values in the different growth phases of pigs more precisely. The project was supported by funds of the Federal Ministry of Agriculture, Food and Regional Identity (BMLEH) based on a decision of the Parliament of the Federal Republic of Germany via the Federal Office for Agriculture and Food (BLE) under the strategy for digitalisation in agriculture, grant number 28DE109E18.

Bone mineral content kinetics in sows across three consecutive lactations as a function of dietary phosphorus during lactation

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Recent data indicate that sows mobilize bone mineral reserves during lactation to meet their calcium (Ca) and phosphorus (P) requirements and subsequently restore these reserves during the following gestation. The mobilization of these reserves is further increased when dietary Ca and P supply is reduced. However, the resilience in bone mobilization-restoration of sows subjected to a low-P lactation diet over consecutive cycles, remains unknown. The objective of the present study was to address this question. A total of 28 primiparous Swiss Large White sows from the Agroscope herd were selected and assigned to four lactation diets formulated to meet the recommended nutrient requirements except for digestible P and Ca: 100 met 100% of the requirement, without exogenous phytase (9.0 g Ca, 3.0 g digestible P/kg, and a 3:1 Ca/digestible P ratio; 8 sows); 50 met 50% of the requirement, without exogenous phytase (4.5 g Ca, 1.5 g digestible P/kg, and a 3:1 Ca/digestible P ratio; 8 sows); 100+FTU (6 sows) and 50+FTU (6 sows) where 100 and 50, were respectively, supplemented with an exogenous phytase (750 FTU/kg, Quantum® Blue 5G, AB Vista, Marlborough, United Kingdom). Sows were fed ad libitum during the lactation. The gestation diet was formulated to cover nutritional requirements and did not contain exogenous phytase. Feed allowance was set at 2.8 kg/day during the first 80 days of gestation and increased to 3.0 kg/day thereafter. Sows were scanned by dual-energy X-ray absorptiometry (DXA) for bone mineral content (BMC, g) and fat tissue mass (kg) over three consecutive lactations (Lact1, Lact2, Lact3) at 2 (d2) and 24 days (d24) postpartum, and during the second gestation (Gest2) at day 40 (d40). The BMC was analyzed using a mixed model including stage (time point of measurement), lactation diet, and their interaction, with a Corsymm residual covariance structure. The mobilized BMC (BMCd24 – BMCd2) within lactation was analyzed using a mixed model including lactation number, lactation diet, and their interaction. In both models, the sow was included as a random effect. A linear correlation between mobilized BMC and fat tissue was performed across the diets 100+FTU, 100, and 50+FTU. On d24 of Lact1, BMC tended to be lower in 50 than in 100+FTU ($P = 0.054$), and on d24 of Lact2 and Lact3, it was lower than in both 100 and 100+FTU ($P < 0.05$). BMC did not differ between treatments at the other stages. The mobilized amount of BMC during lactation did not differ between treatments across lactations. These results confirm that sows fed a low-P diet during lactation mobilized more BMC, and showed that the mobilized BMC was restored over two consecutive gestations. The sow therefore appears to be resilient to a low dietary P supply over several consecutive cycles. The mobilized BMC was linearly associated with the mobilized fat tissue mass ($R^2 = 0.72$, $P < 0.001$). These findings show that during a period of higher metabolic intensity sows mobilize energy from fat tissue and minerals from bone to meet the nutrient demand for milk production. This relationship provides new perspectives for estimating mineral mobilization.

Inter-rater reliability of animal-based indicators intended for organic inspections on pig farms

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Inspections on organic farms must be reliable, and different inspectors should reach the same conclusions when assessing animal welfare. Therefore, animal-based indicators used in inspections need to show a high inter-rater reliability to ensure consistent and comparable results. In the ÖKoTier project, a standardized inspection concept for the annual animal welfare inspection on organic farms is being developed. After selecting suitable animal-based indicators for pigs, the inter-rater reliability was examined in pre-tests between two scientists using photos and videos and subsequently in practice on a sample of sows, suckling piglets, weaned piglets, and fattening pigs. The proportion of agreement, the prevalence-adjusted bias-adjusted kappa (PABAK), the prevalence index (PI) and bias index (BI), and a McNemar test were calculated in R 4.4.1 for the two-score level indicators lameness, manure on the body, tail lesions, ear lesions, skin lesions on the head and carpal joints in suckling piglets, bursitis, body condition score, runts, and hospital pigs. Since the photos and videos were selected based on the criterion that both score levels were evenly distributed, the prevalence effect on these tests was low (PI: median: 0, min: -0.15, max: 0.15). In contrast, the prevalence effect was evident in practice where score 0 generally occurs more frequently than score 1 (PI: median: 0.91, min: -0,24, max: 1). Median PABAK of all indicators tested with photo and video material was 0.9 (min: 0.7, max: 1) and 0.97 (min: 0.52, max: 1) in practice. For indicators with PABAK < 0.81 (manure on the body, skin lesions on the head and carpal joints, runts), an error analysis was performed based on differently rated photos and videos. In addition, systematic differences between the raters were examined using the results of BI (photos and videos: median: 0, min: -0.05, max: 0.06; practice: median: 0, min: -0.11, max: 0.12) and McNemar tests. However, the latter was only significant in practice for the indicator runts in weaned piglets. These results were discussed among the raters, after which some indicator methods were simplified or supplemented with additional information. Subsequently, the inter-rater reliability for these indicators was retested, reaching a good reliability for all indicators in practice (PABAK ≥ 0.8). By testing inter-rater reliability using photos and videos in addition to practical tests, potential prevalence effects can be mitigated. This, together with the calculation of several reliability parameters, enables a comprehensive problem analysis and interpretation of results in order to improve reliability in practice.

Turning Carasau bread leftovers into piglet gains: performance and diarrhoea outcomes after full wheat replacement in the diet

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Sustainability in pig production increasingly depends on improving feed resource efficiency and reducing competition with human-edible ingredients. Circular feeding strategies, valorizing food industry by-products as feed, can contribute to this goal by reducing food waste. The bakery sector generates substantial waste streams, and bread leftovers may offer an alternative to conventional cereals such as wheat in pig diets. However, baking alters starch structure and may influence digestibility, metabolic responses, and diet consistency, which is particularly relevant in the sensitive post-weaning period. This study evaluated whether complete replacement of dietary wheat with flour from Sardinian carasau bread leftovers affects post-weaning piglet performance and health. In a feeding trial, 100 piglets (females and barrows) from 9 sows were used. Piglets from 5 sows were assigned to the control diet (C), a conventional post-weaning diet formulated according to Swiss recommendations (Agroscope, 2025) containing up to 30% wheat, and piglets from 4 sows to the Bread diet (B), in which the wheat fraction of diet C was fully replaced by carasau bread leftover flour. From 21 days of age, piglets had ad libitum access to their assigned feed (creep feed), and after weaning they were allocated to 9 pens per treatment (6-7 pigs/pen), balancing sex ratio as far as possible. Growth and feed intake were monitored up to 7 weeks post-weaning, and diarrhea occurrence was recorded. Mixed-effects models were used for repeated measures on body weight (BW) and average daily gain (ADG), pen-level feed intake, and a binomial model for diarrhea probability. The BW were similar early post-weaning but diverged over time (diet × time, $P < 0.01$), with C pigs being heavier at week 7 than B pigs (27.6 vs. 25.6 kg). Weekly ADG showed a significant diet × week interaction ($P < 0.01$), and overall ADG was slightly higher in C than B (0.41 vs. 0.37 kg/d; $P = 0.04$). Feed intake differed from week 4 onward (diet × time, $P < 0.01$), resulting in lower total intake in B than C (28.9 vs. 33.3 kg/pig; $P < 0.01$) alongside a modest improvement in gain-to-feed ratio for B (0.63 vs. 0.59; $P = 0.05$). Importantly, the B diet reduced the probability of diarrhea compared with the Control ($P < 0.01$). Overall, replacing wheat with carasau bread leftovers supported acceptable post-weaning performance, improved feed efficiency, and lowered diarrhea risk, though with a small reduction in final BW. These findings provide evidence to support bakery by-products as a practical component of circular feeding systems in pig production, while highlighting performance trade-offs that should be considered when implementing full cereal replacement strategies. This work was funded by Ministero delle Imprese e del Made in Italy, Fondo per la Crescita Sostenibile – Accordi per l'innovazione di cui al D.M. 31 dicembre. Project n. F/310035/01-04/X56 "Tecnologie ICT e dell'Industria 4.0 per l'analisi e l'ingegnerizzazione di Sistemi alimentari complessi per la produzione di pani artigianali locali ad alto valore aggiunto (AISAC)

Detection and monitoring of several salivary heat stress biomarkers in growing pigs

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Thermal stress (TS) is one of the main challenges for the pig industry, as it negatively affects animal welfare and productive performance. Its assessment through plasma biomarkers can provide a consistent and objective tool for its detection and correction. However, plasma sampling requires blood extraction, which is inherently stressful. The objective of the present approach was to detect in saliva heat stress-indicators, as alternative biological fluid. Fifteen pigs (Pietrain-Yorkshire x Duroc-Landrance; 35 kg LW) were used and distributed into 3 pens (5 pigs each). After a 7-day adaptation period, animals were subjected to the following environmental conditions in consecutive 7-day periods: Thermal Neutrality (≈23ºC, ≈34% temperature and relative humidity (RH), respectively), TS (≈33ºC, ≈36% RH) and a return to Thermal Neutrality. Rectal temperature was measured every 48 h. Saliva samples were taken on day 7, 14 and 21 of the experimental period. Pig weight, feed intake, and water consumption were daily recorded. Saliva collection was designed to cause minimal disturbance. Disposable sponge held by Kocher forceps were introduced into the pig's mouth and allowed to be chewed. Once saturates with saliva, the sponge was removed and placed in Salivette® vial (Sarstedt, Ref. 51.1534, Germany) and immediately centrifuged (2.500 rpm, 10 min). The analysed biomarker included acute phase proteins (Haptoglobin, Serum amyloid A, C-reactive protein, Apolipoprotein A1, Chromogramin A and Pig-MAP). Specific proteotypic peptides were used as markers for each protein. The proteotypic peptides were analysed by a UPLC-SRM/MS system. Pig performance showed no major residual variability throughout the experimental period compared with standards defined for this breed and age. However, thermal stress significantly reduced dry matter intake and growth performance ($P<0.05$), while water consumption significantly increased (up to 30 %, $P<0.05$) during the hyperthermic phase. Rectal temperature during thermal Neutrality (39.07± 0.30 $_{o}$C) increased significantly under hyperthermia (39.36 ± 0.32 $_{o}$C) and returned to baseline values during the recovery phase (39.02± 0.28$_{o}$C). This may indicate that young pigs were unable to fully adapt to the imposed heat conditions. Regarding salivary biomarkers, Pig-MAP, Chromogramin A and Haptoglobin were not detected in saliva samples, possibly because their concentrations were below the detection limit or because salivary glands may act as a physiological filter for these proteins. In contrast, Apolipoprotein A1 and serum amyloid A were consistently detected in saliva, with concentrations clearly above the detection limit in our UPLC-SRM/MS (in the order of µg/mL). Moreover, a significant reduction in Apolipoprotein A1 (and a trend for serum amyloid A) was observed during hyperthermia, followed by partial recovery after returning to thermal neutrality, although without fully reaching the initial baseline values. C-reactive protein was also consistently detected in saliva across all thermal phases, with concentrations above the detection limit; however salivary C-reactive protein levels remained constant regardless of thermal condition ($P>0.05$). Saliva concentration of the tested biomarker seems to be less sensitive than plasma although the moderate intensity and short duration of the thermal challenge may not have been enough to trigger a marked acute phase response. Additionally, residual variability associated with the sample collection and saliva composition may have introduced biological noise, limiting the statistical significance of some observed differences.

From heat stress to genetic resilience: addressing seasonal infertility in Pigs

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Seasonal fluctuations in environmental conditions, particularly elevated temperatures, present significant constraints to pig production globally. Heat stress adversely affects animal welfare, increases disease susceptibility, and induces endocrine and metabolic disruptions that impair reproductive performance. Due to pigs' limited capacity to dissipate metabolic heat, production losses occur even in facilities equipped with cooling systems. Reproductive traits are especially vulnerable, with heat stress linked to delayed puberty, reduced estrus expression, lower farrowing rates, increased pregnancy loss, and smaller litter sizes, a phenomenon commonly referred to as seasonal infertility. Considerable variation in response to heat stress among animals suggests the presence of genetic differences in tolerance or susceptibility, indicating that seasonal infertility may have a heritable component and could potentially be mitigated through selective breeding. However, the extent of this genetic contribution and the most suitable statistical framework to capture genotype-by-environment (G×E) interactions and environmental sensitivity remain uncertain. Canadian (N = 391,275) and Brazilian (N = 544,865) datasets will be analyzed to represent contrasting climatic conditions (temperate vs. tropical) from 2016 to 2025. The study includes two purebred maternal lines (L1 and L2), enabling the evaluation of animals across distinct environmental gradients. Evaluated traits will include total number born, total number of stillborn, farrowing rate, and gestation length. Preliminary population statistics showed differences in maternal lines across countries and seasons. In Canada, reproductive performance for L1 was slightly lower in summer than in winter, with fewer total born (13.92 vs. 14.08), fewer stillborn (0.96 vs. 1.03), and a shorter gestation length (117.5 vs. 118.1 days). Farrowing rate remained stable (99.29% vs. 99.23%). For Line L2, seasonal variation was minimal in total born (15.27 vs. 15.86), stillborn (1.28 vs. 1.32), and gestation length (115.3 vs. 115.4 days), though farrowing rate was slightly lower in summer (98.05% vs. 99.43%). In Brazil, both lines showed high seasonal stability. Line L1 had minimal variation in total born (14.28 vs. 14.29), stillborn (0.74 vs. 0.70), and gestation length (115.9 days), with a slightly lower farrowing rate in winter (99.82% vs. 99.98%). Line L2 remained consistent across seasons for total born (15.13 vs. 15.14), stillborn (0.99 vs. 0.98), and gestation length (114.5 vs. 114.6 days), with identical farrowing rates (99.9%). It is hypothesized that variation in seasonal infertility also has a measurable genetic component and that selection for greater robustness, as reflected by improved mean performance and reduced environmental variability under heat stress, is feasible. To test these hypotheses, random regression models across environmental gradients, multi-trait models across countries and gradients, and Double Hierarchical Generalized Linear Models will be compared to quantify environmental sensitivity and genetic heterogeneity of residual variance, and to determine which model best captures the influence of environmental factors. By integrating diverse statistical approaches and contrasting production environments, this study aims to clarify the genetic architecture underlying seasonal infertility and to evaluate the potential benefits of incorporating heat tolerance and robustness into commercial breeding programs, thereby supporting more sustainable pig production in the context of ongoing climate change.

Reframing sustainability for a fairer future for livestock farming: A One Welfare approach

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One Welfare highlights the interconnections between animal and human welfare within their shared environment. Post-war agricultural development prioritised yield and cost (i.e. economic sustainability) at the expense of broader concerns. The three-pillar model (economic-social-environment) emerged to correct this imbalance. However, environmental sustainability is narrowly fixated on GHG emissions and technological solutions, sidelining other environmental issues (e.g. biodiversity loss), while social sustainability remains marginalised. Rising production costs and price volatility drive continued dominance of the economic pillar and with-it the continued externalisation of costs onto people, animals, and the environment, interconnected "One Welfare" burdens. This paper presents a case for re-framing sustainability around One Welfare to ensure a fairer future for livestock farming. We undertook an ad hoc review of the livestock sustainability literature to; 1) provide evidence of the conflicts and weaknesses of current frameworks, 2) an overview of overlap between economics and social sustainability and 3) to investigate an approach to sustainability that focuses on human and animal welfare underpinned by a healthy shared environment. We found that currently, animal welfare as part of the social pillar is almost entirely neglected and is sidelined when it conflicts with environmental objectives. There is growing acceptance that animal welfare should be considered separately. Ideally this would ensure that trade-offs, like when animal welfare conflicts with nitrogen use efficiency, are evaluated in a balanced manner. Nevertheless, despite human welfare being long represented by the social pillar, this dimension is consistently marginalised. for example, the healthiest diets for humans being seemingly carbon inefficient are eschewed in favour of plant based or highly processed meat alternatives (e.g. EAT-Lancet Commission report). This indicates that a dedicated pillar in the sustainability framework does not guaranty fair representation in the sustainability debate. There are calls for a conceptual sustainability framework based on integrity in which sustainability conflicts could be addressed by prioritising the welfare of humans and animals, as sentient beings, over non-sentient concerns (e.g. GHG emissions). The One Welfare concept of interdependence means that improving animal welfare leads to better human welfare, and vice versa, and that both rely on a healthy shared environment. Hence, non-sentient concerns would be addressed by default within holistic, often nature-based solutions rather than as singular technological solutions. Under economic sustainability, scale and efficiency are prioritised to the benefit of corporate players. Other sustainability frameworks position economics within the social pillar (e.g. FAO SAFA) ensuring that they have a higher human value (e.g. wages and prices) and are therefore economically fairer. This is akin to the One Welfare concept where economic benefits to society are outcomes of good human, animal and environmental welfare. Animal welfare requires a dedicated sustainability dimension to ensure the future of livestock production. Positioning economics within the social dimension requires it to serve human welfare, over corporate needs. Environmental sustainability remains crucial, but it should never trump the welfare of humans or animals (wild or domesticated) and within One Welfare it is protected by default. This reframing of sustainability using the One Welfare framework acknowledges that while there are trade-offs, putting sentience, and therefore the welfare of people and animals first, positioned within a shared healthy environment offers a fairer future for livestock farming.

Aggregation of indicators for the multifunctionality of mountain dairy cattle farms: a One Welfare approach

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There is growing recognition of the need for integrated sustainability assessment tools that go beyond traditional environmental metrics to capture the complexity of livestock systems. The literature highlights the diversity of sustainability indicators proposed for dairy farms across the European Union and emphasises the importance of including animal welfare alongside agroecological, social, and environmental dimensions in order to support decision-making and policy development. Therefore, the objective was to develop a Multifunctionality sustainability Index (MInd) which aggregates multiple indicators to support the evaluation of mountain dairy farms' sustainability within a One Welfare approach. The study involved 20 mountain dairy farms and integrated 20 indicators encompassing animal welfare (W), agroecological (AE) and socio-territorial (ST) dimensions (based on IDEA4 farm sustainability indicators), as well as environmental impacts (I; based on Intergovernmental Panel on Climate Change). Data were collected through legal documents, the Italian Breeders Association, visual inspection and a questionnaire to farmers regarding all aspects of farm management. The MInd was built and then applied to farms. When several indicators exhibited high correlations (>0.8), an intermediate principal component analysis was conducted to select a single representative indicator. The MInd consisted of 9 key indicators in the final model, calculated as follow: MInd = + 0.011 * behaviour (W) + 0.385 * functional diversity (AE) + 0.227 * closed-loop material and energy flows in pursuit of autonomy (AE) + 0.390 * frugality in the use of resources (AE) + 0.340 * reduction of the impact on human health and ecosystems (AE) + 0.314* food production(ST) + 0.282 * local development and circular economy (ST) + 0.431 * employment and quality of work (ST) – 0.410 * ecotoxicity (I). MInd is mainly driven by employment and quality of work (0.431) and by ecotoxicity that showed a negative loading (−0.410), meaning that higher environmental impact reduces the index value. MInd successfully integrates all the initially intended dimensions and allows for differential weighting of each indicator, reflecting their relative contribution to overall sustainability. MInd offers a practical approach to monitor progress, identify strengths and weaknesses, and guide policies aimed at fostering sustainable and resilient dairy systems in mountain areas. This work was carried out within the project 'Looking for the agro-environmental friendly dairy farm: innovative approaches and integration of methodologies for an inclusive definition of sustainability in dairy systems', funded under the 2022 call by the European Union – NextGenerationEU.

Integrating Positive Animal Welfare and One Welfare: A Socio-Environmental Framework for Sustainable Livestock Systems

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Farm animal welfare science has traditionally focused on preventing unnecessary suffering and mitigating negative affective states in animals. However, sustainable livestock systems require moving beyond deficit-based models toward approaches that actively promote positive experiences and account for broader socio-ecological dynamics. Positive Animal Welfare (PAW) emphasizes animals' positive affective states and experiences, whereas One Welfare highlights the interconnections between animal welfare, human wellbeing, environmental sustainability, and socio-economic viability. Despite increasing joint use, their conceptual integration remains insufficiently defined. We hypothesise that animals' opportunities to experience positive states, and the long-term sustainability of livestock systems, are systematically shaped by socio-ecological conditions at farm level, including management practices, human–animal interactions, labour conditions, environmental constraints, social acceptability, and economic profitability. Integrating PAW within a One Welfare approach could promote welfare improvements while positively impacting farm sustainability. This contribution, developed within the EU COST Action LIFT (Lifting Farm Animal Lives – laying the foundations for positive animal welfare, CA21124), aims to present an operational framework that aligns animal-based indicators of positive experiences (e.g. play, exploratory, affiliative behaviours and affect balance) with system-level variables. By combining fine-grained measures of individual experience with relational, system-level analysis, the framework may provide a scientific basis for linking measurable positive welfare states to the conditions that enable or constrain them. It may support the development of transferable, context-sensitive assessment tools and practical strategies that are socially acceptable and economically viable. Ultimately, this work will clarify the complementary roles of PAW and One Welfare, contributing to the embedding of positive welfare science within real-world livestock production systems.

Bringing animal welfare into sustainability: embedding animal wellbeing in Life Cycle Assessments

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Animal welfare is an essential but often underrepresented dimension of sustainability in livestock production. Although sustainability frameworks—including the United Nations Sustainable Development Goals and conventional Life Cycle Assessment (LCA)—address environmental, economic, and social aspects, they provide limited recognition of welfare outcomes within food systems. Given the close interconnections between welfare, environmental performance, human health, socio-economic performance and resilience, and societal acceptance, this study aims to articulate a coherent pathway for integrating animal welfare into holistic sustainability assessment. We identify conceptual and methodological gaps in current frameworks and proposes recognising animals as a distinct stakeholder group within Social Life Cycle Assessment (S-LCA). We outline a set of candidate activity variables suitable for quantifying welfare impacts, including time-in-condition, behavioural opportunity indicators, exposure to stressful interventions, health and vitality metrics, and cumulative welfare scores. A central methodological proposal is the use of "animal life days" as a scalable, functionally compatible activity variable that captures the duration animals spend in defined welfare states across production systems. This is equivalent to the notion of 'load', i.e. how long animals spend in the presence of a stressor. Adopting "animal life days" and related welfare metrics enables direct comparison of welfare outcomes across livestock systems and facilitates integration into established S-LCA impact pathways. The approach supports quantitative evaluation of welfare alongside environmental and socio-economic impacts, preserving the holistic intent of sustainability assessment. It also strengthens ethical coherence, enhances transparency in trade-off analysis, and aligns assessment practice with increasing societal expectations for responsible livestock production. We will provide a hypothetical example of how the concept of 'animal life days' would apply to the comparison between two systems that differ not only in the prevalence of welfare issues but also in the duration of exposure and the space available to each animal which determine likelihood of exposure. Embedding animal welfare within S-LCA provides a systematic and practical means to advance socially responsive, ethically grounded sustainability assessment in food systems. Recognising animals as stakeholders and operationalising welfare through measurable activity variables can support more credible decision-making for producers, integrators, policymakers, and consumers. Integrating welfare into existing LCA-based frameworks thus offers a pathway toward livestock systems that are not only environmentally efficient but also ethically and socially robust.

Development and on-farm implementation of a multidimensional sustainability assessment and benchmarking tool for pig and broiler production

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Sustainable animal production is a multidimensional concept encompassing animal welfare, environmental impact, economic viability and social well-being. Despite increasing societal and policy attention, livestock farmers often lack clear insight into their overall sustainability performance. Most existing frameworks focus on a single sustainability aspect –typically economic and ecological impact– leaving animal welfare and social dimensions underrepresented. Given that improvements in one dimension can entail trade-offs in others, a holistic perspective is essential for informed decision-making. To address this gap, we aimed to develop a multidimensional sustainability assessment, information and benchmarking tool for pig and broiler production farms. The tool provides structured insight into farm performance across the 4 key sustainability pillars: animal health and welfare, environment, economy and social well-being. The online survey with automatic and personalized reporting was developed and evaluated in close collaboration with farmers, experts and stakeholders. Within each sustainability pillar, a set of carefully selected key performance indicators (KPIs) was defined, based on existing questionnaires and tools (e.g. AMCRA, Ruckli et al., 2022) as well as expert discussions. This resulted in 9 KPIs for animal health and welfare, 8 for environment, 9 for economy and 8 for social well-being. The survey entails 56 questions and takes between 30 to 90'. The report consists of 3 parts: 1) global overview figure of the two best and least scoring KPIs per sustainability pillar, 2) summary figure for each sustainability pillar presenting the scores of all KPIs, categorized into strengths and areas for improvement, for which recommendations are provided, and 3) more detailed information for all KPIs, including benchmarking across all participating farms. Rather than providing a weighted average that might mask underlying issues, the tool focuses on identifying specific strengths and critical points of attention for each farm. By evaluating the KPIs against predetermined reference values, relative scores are calculated. This results in a personalized report with targeted recommendations corresponding with those indicators requiring attention. Additionally, the tool fulfills a significant industry demand for benchmarking, allowing farmers to compare their results with peer groups, thereby providing an extra incentive for adoption. The applicability and suitability of the tool were evaluated through implementation on 33 pig farms and 23 broiler farms in Flanders. In general, pig farms obtained high scores for the KPIs feed conversion ratio, mortality rate and barn insulation, whereas lower scores were observed for sustainable water use. High variability was observed for most economic KPIs (e.g. family labor income and labor productivity) and for total energy use. Broiler farms generally scored high on liquidity, biosecurity and dead-on-arrival, while lower scores were observed for profitability and sustainable water use. Similar to pig farms, high variability was found for most economic KPIs (e.g. feed cost) and for renewable energy use. For the social dimension, both pig and broiler farmers generally scored well, with the highest scores achieved for management and access to advice and knowledge, whereas average scores were lower for networking and work-life balance. The results demonstrate the feasibility of a multidimensional, non-aggregated sustainability assessment approach and highlight the variability in performance both within and across sustainability pillars.

One Welfare : Perception and expectation by French representative of beef and dairy cattle sector

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Context and Objective. This study was part of the MICOW project which addresses the challenge of turning the "One Welfare" concept—linking animal welfare, farmers' well-being, and the environment—into a practical support method for French cattle farms. It starts from the observation that livestock farmers face growing, sometimes conflicting demands (productivity, regulations, societal expectations), and asks how a One Welfare–based approach could help in real farm conditions. The study's core questions focus on how diverse cattle-sector stakeholders understand One Welfare, what they expect from advisory/support methods, and how such a method could be operationalized in French beef and dairy systems. Methodology. The work followed a qualitative design conducted from March to August 2024. After an exploratory phase (literature review, context building, and exploratory interviews), we carried out 26 semi-structured interviews with a wide range of actors across French cattle value chains (e.g., advisory services, interprofessional bodies, technical and research organizations, veterinarians, and farmers). Interviews were recorded, transcribed, and analyzed through thematic content analysis (to identify recurrent ideas, tensions, and expectations) and typological analysis (to build stakeholder profiles based on their level of adherence to the One Welfare concept and willingness to implement it operationally). Results. Stakeholders broadly agreed that farmers' well-being and animal welfare are interconnected, but they differed in how they define animal welfare and how "One Welfare" should be interpreted. Many actors framed animal welfare using established references (e.g., "Five Freedoms" and ANSES' definition) while debating whether these sufficiently capture animals' emotional and individual dimensions. In contrast, farmers' well-being was harder for interviewees to define precisely, yet perceptions converged around physical and psychological comfort, workload, recognition, income, and the ability to rest. One Welfare was widely seen as promising but often "too conceptual," with many actors focusing mainly on the human–animal relation and leaving the environmental dimension less explicit. Operationalization proposals clustered around four formats: collective farmer groups, training advisors/technicians in a One Welfare approach, integrating One Welfare elements into existing diagnostic tools, and self-assessment tools for farmers. A typology identified four profiles—from highly supportive "enthusiasts" to skeptical actors who viewed implementation as too complex in terms of skills, time, and funding. Conclusion. We conclude that One Welfare has strong perceived relevance for supporting cattle farmers under current pressures, but its practical deployment requires clearer framing, shared tools, and coordinated multi-actor implementation. The study highlights the need for pragmatic, farm-adapted methods that avoid being perceived as accusatory, and for credible incentives and funding mechanisms. It also recommends further work that directly includes a broader, more diverse sample of farmers (beyond organizational representatives) and possibly explores other livestock sectors, while improving communication to align field interpretations of One Welfare with its more comprehensive academic definition.

One Welfare on a Family Beef Farm: Human–Animal Co-Agency Driving Environmental Adaptation and Relational Innovation

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Beef suckler systems depend on dynamic interactions between humans, animals, and environmental conditions, shaping welfare and agency. Within One Welfare, family farms offer a relevant context to analyse how farmers and cattle act as co-agents adapting to climatic constraints, managing landscape features, and resource availability. In such systems, the present study aimed to describe work organisation, innovation, and collective decision-making. It also aimed to identify adaptive practices triggered by environmental or behavioural cues. At last, it aimed to highlight risks and perspectives for improving safety, resilience, and sustainability. A qualitative case study was conducted on a family farm that transitioned from dairy to Limousine cattle 30 years ago. Semi-structured interviews and thematic analysis focused on routines, infrastructure, grazing management, and human–animal interactions. In this farm, work organisation is collective, anticipatory, and environmentally informed. Decisions on breed, grazing, or buildings are discussed within the family and supported by peer exchanges and training. Innovation is pragmatic: enrichment devices, ventilation changes, or building adjustments are tested before adoption. Farmers maintain a critical opinion toward costly solutions lacking clear justification, preserving human agency in decision-making, and economic viability. Environmental factors such as wind exposure, soil moisture, drought, humidity, water availability and shelter distribution play a central role in daily management. Farmers observe how animals select resting or grazing areas accordingly. These choices guide paddock rotation, building design, and circulation routes. Climate variability requires constant adaptation: windbreaks, adjusted wall protections, improved airflow and light, managed transitions between pasture and buildings, and maintenance of clean water points. Environmental mismatches can lead animals to leave inadequate pastures, coming "under farmers' windows" for signalling the need for rapid corrective action. Pasture quality and winter shelter strategies require yearly revision based on climatic trends and observed behaviour. Cattle are considered active partners influencing management through preferences, social dynamics, and behavioural signals. Farmers adapt by avoiding isolation of primiparous heifers, mixing age groups to support social learning, modifying water access when dominant animals block troughs, and providing simple enrichment to reduce fear and limit building damage. Calm handling, ritualised visits to calves, and culling of animals with poor temperament support human safety and animal emotional stability. This case study shows how One Welfare emerges through environmental and relational practices where humans and animals co-construct their shared environment. Innovation is grounded in routine decisions, and the family's critical evaluation of external advice reinforces autonomy and resilience. The system is continuously evolving, illustrating how family farms operationalise the One Welfare approach. This study was supported by the Carnot Micow Project.

How to balance three welfare dimensions in dairy farm management

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This study is based on the EU-project Resilience for Dairy, which dealt with challenges for the future facing the sector. The project aimed to develop a network on resilient dairy farms in the participating 15 countries during a 4-year period. On basis of statistical data, it is argued that size of farm (20-150 cows) has only a limited correlation with family income. Hitherto, management capacity is a dominant factor for farm viability. This presentation aims to examine how dairy farm management can balance three welfare dimensions – farmers' welbeing, animal welfare and environmental sustainabilitity – to support resilient long-term stategies. To support management, farmer needs and solutions, i.e. practices and techniques, were systematically gathered and assessed. Farmer needs were captured through European experts' meetings and in regional stakeholder workshops, resulting in a list of 43 categories of needs (rated on a scale from no contribution to great contribution to resilience) by using an online questionnaire. 535 stakeholders in the participating countries (70% farmers, 30% other stakeholders) scored the list of needs. Three key know-how fields emerged: the need for economic/ social resilience, technical efficiency, and adaptation to environmental and animal welfare standards. Next, 190 solutions – practices and techniques) – were chosen linked to these know-how fields on their expected contribution to farm resilience. An online survey assisted in assessing each solution separately; 3329 assessments were done with on average 53 solutions per expert assessor. Additionally, stakeholder workshops across Europe chose and ranked the 20 most urgent solutions in their country to strengthen farm resilience. Results indicated regional variation in needs across Europe (Western Europe vs Central and Eastern Europe) with differences related to technical efficiency, knowledge development, and environmental and animal welfare practices. Younger farmers tended to mention socio-economic aspects as income and work-life balance more often than older farmers. However, it was also noted that year-to-year issues which raise a lot of attention affected needs, like in Denmark by the introduction of a CO2-tax for dairy farms; in the Netherlands by the so-called N-crises, that shifted needs to more interest in soil health and quality; and in Slovenia by the assignment of NGO's to check on-farm for lack on animal welfare. From this study emerged the question how on farm level a trade-off between socio-economic welfare including communication with society (farmers' welfare), animal welfare, and environmental sustainability/welfare takes place to arrive at a resilient future farm business. Which factors affect a sound balance and strategy between these 3 x welfare components and is there a methodology available to support the management and manager in this context? This presentation will address these questions. As an example, an interactive strategic management program will be outlined in which the farm family develops its own plans in cooperation with experts and peer farmers, explicitly considering synergies and trade-offs between the three welfare dimensions. Keywords: 3 x welfare, farmers' welbeing, animal welfare, environmental sustainability, workshops, strategic management, future farm

Balancing three pillars of welfare to maintain a promising future for dairy farming in the USA

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To maintain a promising future, dairy farms in the USA need to meet the three pillars of welfare, be resilient and profitable. Human welfare relates mostly to human resource management in the USA. Dairy herd sizes have increased greatly in the last 20 years. The larger herd sizes of the remaining dairy farms have caused many farms to rely on hired outside labor. Many workers come from Latin America. As a rule of thumb, dairy farmers with more than 300 cows become human resource managers, rather than doing much of the physical work themselves anymore. Dairy farm labor management has been studied, practiced, and refined for over decades now. It receives constant attention at meetings for dairy farmers. Where two decades ago the topic of cow comfort received much attention, today there is at least equal attention on maintaining productive and happy workers willing to work hard for long hours. This varies from offering good pay, bonuses, housing, training, to safety regulations, respect and cultural awareness. Almost without exception, well-run and profitable dairy farms have excellent labor management. Because dairy farm workers are scarce, in part because the immigration policy is reducing the supply of foreign workers, there is much interest in improving labor efficiency and robotics to get more work done with fewer people. Thus, larger dairy farms in the USA have mastered human resources management in similar ways to midsize firms in other sectors that employ workers. Animal welfare is practiced primarily because it's seen as the right thing to do and makes business sense. There are animal welfare laws in the USA, but they're limited, fragmented, and mostly state-driven, especially compared to Europe. One example of a federal law is the humane slaughter act. Regarding conventional dairy farming, there are no laws about, for example, pasture access, housing or stocking density. Animal welfare is more addressed in private programs such as the National Dairy FARM (Farmers Assuring Responsible Management) Program. More than 98 percent of the milk supply now comes from farms participating in FARM Animal Care. It is common now to find cow stockmanship training and monitoring of the way cows are treated to ensure cow welfare. Modern dairy barns include free stalls with ample air, feed, water, and a clean, soft, well-sized place to lie down. Human and animal welfare are connected through an emphasis on comfort for both the worker and the cattle. However, special care of dairy animals is limited because of the business approach to dairy farming. Environmental stewardship is not labeled as "welfare" in the USA. Environmental stewardship is more regulated than animal welfare, for example through the federal Clean Water Act, manure handling rules, and, in some states, regulations about air emission. In addition, the public is demanding a commitment from the dairy sector to reduce dairy's carbon footprint, conserve water resources, promote water quality, and protect biodiversity. Examples of stewardship are the quantification of dairy's impact on the environment, and commitments to reduce it such as through research to obtain more environmentally friendly cows. In a broader sense, environmental stewardship also includes the communities of people where dairy farms are located. There is an awareness that dairy farms need to be community partners and be known for providing nutritious, healthy products. These three pillars of welfare are necessary but not sufficient for a promising future because dairy farms also need to remain profitable and able to absorb shocks to the dairy business.

Integrating Animal Welfare into Sustainability Assessment of Livestock Systems: A Scoping Review and Expert Consultation

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Animal welfare is key to sustainable livestock production, yet its integration into sustainability assessment frameworks remains fragmented. This study aimed to i) map current knowledge on animal welfare integration, ii) identify welfare indicators and their operationalisation, iii) examine methodological challenges, and iv) elicit expert perspectives on optimal approaches. A scoping review (2015-2025) identified 26 studies integrating animal welfare into sustainability assessment across livestock species. Data extraction captured framework types, indicator categories, measurement scales, and welfare domain coverage of the indicators using the Five Domains Model. A focus group workshop with 15 animal welfare scientists, Life Cycle Assessment practitioners, and sustainability researchers addressed five research questions: i) indicator selection, ii) measurement challenges, iii) framework positioning, iv) trade-off handling, and v) future improvements. Seven framework types were identified, with multidimensional sustainability (27%) and conceptual frameworks (27%) most prevalent. In terms of approaches, management/resource-based proxies dominated (42%), while animal-based measures comprised only 27% despite being more direct welfare assessments. Welfare domain coverage of the indicators showed strong coverage of health (77%) and physical environment (69%) but limited mental state coverage (35%). Expert consultation revealed unanimous agreement that animal welfare is essential to sustainability, yet with divergent positioning views: integral component (47%), social pillar (18%), separate fourth pillar (24%), or cross-cutting (12%). Six major challenges emerged: data availability and scalability, functional unit(s) selection, lack of standardisation, measurement validity, context-dependency, and aggregation and weighing issues. Experts prioritised standardisation, databases, technological innovation, and framework integration. This study reveals substantial methodological diversity and limited consensus on animal welfare integration. Limited mental state coverage of the indicators shows that current frameworks predominantly assess physical welfare while neglecting affective experiences, which is the ultimate welfare determinant per the Five Domains Model. Unresolved questions regarding positioning, weighting, functional units, and valuation reflect philosophical tensions about animals' moral status. Progress requires: i) standardised indicators spanning all welfare domains including mental states, ii) comprehensive databases for routine assessment, iii) transparent value-based weighting engaging scientists and stakeholders, and iv) revision of international frameworks to explicitly accommodate animal welfare in livestock sustainability assessment.

Interrelationships between animal welfare and sustainability of on-farm best practices for poultry production

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Multiple practices are applied in broiler and laying hen production to improve animal welfare. Such practices may also have impacts on the environmental, social and economic sustainability dimensions, either positive (synergies), negative (trade-offs) or neutral. Within the action "Sustainability aspects of animal welfare-promoting livestock systems" of the European Partnership on Animal Health and Welfare (EUPAHW), a scoping review was conducted to map interrelationships and identify knowledge gaps between welfare improvement and other sustainability dimensions. This was applied on a selection of best practices improving animal welfare at the farm stage: "Outdoor access", "No use of cages", "Lower stocking density", "Lower growth genetics", "Microclimate management", "Good litter management", "Enrichments". Overall, there was an important imbalance in the number of articles found depending on the practice and dimension assessed. For economic sustainability, direct costs were often not displayed, and the dimension was envisioned through production parameters (egg laying rate, slaughter weight, feed conversion ratio, etc.). It was difficult to draw conclusions from best practices such as enrichments, where the economic impact varied greatly depending on the type of enrichment used. However, some practices clearly appeared to worsen economic sustainability, such as the use of non-cage systems, while other improved it, such as microclimate management. For environmental sustainability, quantitative assessments of environmental footprints were found for all practices but enrichments, though the indicators studied were not always the same (for instance, ammonia emissions were common while land use was less calculated). Apart from microclimate and good litter management, the environmental impacts were mostly negative. Regarding social sustainability, meat quality was the most studied, with impacts varying greatly depending on the conditions of the practices (e.g., strain). Consumers surveys were recurring, though results varied greatly across countries. The surveys usually addressed priorities when buying products, revealing contradictions between the willingness to improve animal welfare and to pay, and the act of purchase. Farmer surveys, especially on working conditions, were described in the literature for about half of the practices. Overall, this scoping review identifies knowledge gaps on the impacts of animal welfare best practices on sustainability dimensions. It also highlights the lack of tools and methods for complete and holistic sustainability assessments including animal welfare, allowing comparisons across studies and practices. This work was co-funded by the European Union's Horizon Europe Project 101136346 EUPAHW. Views and opinions expressed are those of the author(s) only and do not necessarily reflect those of the European Union or the European Research Executive Agency. Neither the European Union nor the granting authority can be held responsible for them.

Rational and Sustainable Rabbit Farming in Burkina Faso for Food Security and Income Creation for Vulnerable Populations: The SOAMBA project

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Widespread hunger and poverty continue to characterize many Least Developed Countries in sub-Saharan Africa, reflecting a persistent failure in equitable food access and rural development. Addressing these challenges requires context-specific strategies grounded in local environmental and socio-economic realities. In West Africa, Burkina Faso faces climatic constraints, resource limitations, and structural vulnerabilities affecting agricultural productivity and food security. In response, the Italian Agency for Development Cooperation (AICS) has funded the SOAMBA project, contributing to SDG 2 (Zero Hunger) by strengthening the rabbit-meat value chain through integrated research–training–development framework. The project is led by the University of Perugia (Unipg) in partnership with Tamat ETS and five Burkinabé partners: The National School of Animal Husbandry and Health (ENESA), the Institute for Environment and Agricultural Research (INERA), the Association of Producers of Livestock Sectors in Burkina Faso (AFA BF), Association Manegdbzanga, and the Patronale Association of Hotels and Restaurants of the BF (APHRBF). The project is establishing two Genetic Centres at ENESA (Boulbi) and the Bangr Baoore Centre in Loumbila, designed as hubs for breeding improvement, research, and technical innovation. Current research activities focus on the characterization and enhancement of local rabbit genetic resources to increase productivity while safeguarding adaptive traits required under Sahelian climatic conditions. The objective is to raise average litter size from approximately five kits to 8–10, increasing annual production per doe. Within an agroecological framework, feeding formulations are being evaluated using local, non-competitive resources and agro-industrial by-products, including mango processing residues, moringa, amaranth, and sweet potato leaves. Circular production principles are reinforced through agroecological practices, including organic mounds fertilized with rabbit manure. Additionally, heat-resistant housing and static refrigeration systems are being prototyped to safeguard animal welfare and meat quality. The sustainability of the initiative relies on a Training-of-Trainers model. Capacity-building courses are being developed on site and in Italy. By embedding knowledge transfer within Burkinabé institutions, the initiative promotes self-sustaining capacity development beyond its timeframe. Training outcomes are evaluated through standardized tools. Selected technicians undertake a residency at Unipg to act as local trainers and innovation catalysts. In parallel, satellite family farms are being developed in Loumbila and Saaba. This model facilitates the scaling-out of rabbit production among rural households as a food security strategy. Beneficiary selection ensures at least 45% female participation and facilitates the inclusion of persons with disabilities. By integrating animal genetics, sustainable housing and feeding, technical capacity, and circular models, SOAMBA operationalizes the One Welfare framework, proposing resilient and locally adaptable pathways to improve food security and livelihoods.

Farmers' Negative Emotions as a Component of the Administrative Burden of Farm Supervision

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Background Administrative burden refers to additional work farmers must carry out for purposes other than their own operations. Such tasks include notification and reporting obligations, permit procedures and support applications. Psychological stress, which includes fear and other negative emotions, is part of administrative burden. Administrative burden may accumulate when a farm faces simultaneous obligations from both the industry and public authorities. Emotions can also be influenced by so-called annoyance effects, which refer to the combination of the comprehensibility of the legal requirements, the ease of implementing the statutory information obligations, and the acceptability of the obligations. Farmers' well-being can be a limiting factor for providing good care for animals; thus, identifying ways to reduce the administrative burden linked to animal welfare supervision and farm subsidies is important. Objectives The aim of this study was to identify risks that may compromise farmers' legal protection and to explore the administrative burdens associated with animal welfare supervision and animal-related subsidies. Methods The research data were collected through thematic interviews of twelve voluntary Finnish cattle and sheep farmers. Before the interview, interviewees received an information brochure and privacy statement, and consent was obtained. The themes covered topics such as subsidies affecting animal welfare, sources of regulatory information, supervision procedures, role of the industry, and farmers' development proposals. Anonymised summaries were analysed using Atlas.ti software using inductive approach. Results The interviews revealed a wide range of negative emotions including stress, fear, a sense of injustice, tension, strain, uncertainty and external pressure. The most frequent risk to legal protection was inconsistent interpretation of regulatory conditions. Uncertainty of interpretations underlied stress and other negative emotions. Factors that can reduce psychological strain included familiarity with the supervisor, farmer involvement in policy design, education, clear legal rules, and considering the long-term perspective of agricultural work. Discussion & conclusion Administrative burdens drain farmers' resources and may risk both farmer and animal welfare. Inconsistency among supervisors' interpretations both undermines legal protection and increases administrative burden. Ethical, trust-building supervision requires that all farmers are treated according to the same rules and guidelines. Farmers need sufficient information to comply with regulations, and providing this information is the responsibility of authorities. While clear instructions help farmers understand their legal position, excessive guidance may lead to information overload, hindering the intended clarity. Broader consultation with farmers would help strengthen animal welfare policies by identifying actions that engage farmers and help reveal potential issues that may give rise to disagreements. Clear communication, customer encounters and interaction play a key role in experience of regulatory burden. The agricultural support system is characterised by constant change: some rules evolve quickly, while others change only slowly. The slowness of change is also partly due to path dependency, and institutionalisation, which means that the solutions made have many advantages, which makes them difficult to dismantle. Steadiness of regulations also support legal certainty. Stress and the risk for burnout can also threaten animal welfare on farms. Sustainable farming must therefore encompass farmer well-being. Psychological burden should also be considered when evaluating administrative burden.

Animal welfare and sustainability of pasture-based dairy production: a Life Cycle Assessment modelling approach

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The sustainability of livestock production is a strategic priority for European agriculture. Within this framework, animal welfare (AW) is increasingly recognised as a key determinant of both environmental and economic sustainability of farming systems. In the context of the European Partnership on Animal Health and Welfare (EUPAHW), this study develops a Life Cycle Assessment (LCA) modelling approach to evaluate the impact of welfare conditions in pasture-based dairy cows on the carbon footprint of milk production. A targeted literature review was conducted focusing on semi-intensive grazing systems with rotational pasture management. Several welfare impairment conditions were considered, including lameness and hoof disorders, inadequate nutrition and water access, heat stress and lack of shade, parasitic and infectious diseases, injuries, and social competition. For each factor, the effects on productive performance and environmental sustainability were assessed. The carbon footprint was calculated per kg of fat- and protein-corrected milk (FPCM), comparing an optimal welfare scenario (baseline) with different impaired welfare scenarios, considering both single factors and combined conditions. The increase in emission intensity (kg CO_2-eq/kg FPCM) was estimated through the integration of bibliographic data and modelling tools. Preliminary results indicate that compromised welfare conditions at pasture lead to an increase in milk emission intensity compared with the optimal the analysis also highlighted significant knowledge gaps in available datasets, suggesting the need for targeted experimental studies. Overall, the study demonstrates that improving animal welfare can represent an effective climate mitigation strategy in pasture-based dairy systems, aligning environmental and productive objectives. This work was co-funded by the European Union's Horizon Europe Project 101136346 EUPAHW. Views and opinions expressed are those of the author(s) only and do not necessarily reflect those of the European Union or the European Research Executive Agency. Neither the European Union nor the granting authority can be held responsible for them

From preventing suffering to measuring positive welfare: Can biological markers reflect Animals' lifetime experiences?

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Animal welfare science has traditionally focused on identifying and preventing suffering. However, increasing attention is now being given to positive welfare and to understanding whether animals experience a life worth living. Addressing this challenge requires indicators that reflect not only momentary states but also the cumulative balance of positive and negative experiences across an animal's lifetime. Research, not necessarily conducted in non-human or farm animals, suggests that several biological analyses may provide promising indicators of cumulative experiences. These include, for example, changes in brain plasticity, telomere dynamics, oxidative stress pathways, immune function, the gut microbiome, and metabolomic profiles. While many of these approaches have primarily been studied in relation to negative exposures, emerging work indicates that they may also capture aspects of positive experiences and long-term welfare states. In this talk, current research and conceptual developments on biological indicators of positive and cumulative welfare will be reviewed, highlighting metabolomics as an emerging tool for understanding how lifetime experiences become biologically embedded. Although none of these measures currently provides a definitive indicator of positive welfare, they offer promising methodological directions for advancing welfare assessment, particularly when used in combination.

Wool Cortisol Reveals Cumulative Welfare and Physiological Resilience in Sheep Production Systems

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Chronic stress represents a cumulative welfare burden in extensive sheep systems, yet robust longitudinal indicators remain limited. This study evaluates wool cortisol as a retrospective biomarker of long-term welfare across contrasting production environments. Karayaka sheep (n = 75) were monitored over 8 months with repeated wool sampling (8 time points) under intensive housing, short-distance transhumant pastoralism, and long-distance highland pastoralism. All animals were exposed to comparable environmental and management-related stressors within their respective production systems, without the application of differential experimental treatments across subgroups. Wool samples were consistently collected from regrown fleece, reflecting cortisol accumulation over the preceding growth period. Wool cortisol concentrations ranged from 3.22 to 7.00 pg/mg, showing significant production system × season interactions ($P < 0.05$). Short-distance pastoral flocks exhibited the highest mean cortisol (5.49 ± 0.20 pg/mg), with pronounced elevations during winter confinement, indicating welfare challenges associated with abrupt environmental transitions. Long-distance migrating sheep showed transient post-migration stress peaks followed by marked physiological recovery during alpine grazing (July: 3.33 ± 0.20 pg/mg), suggesting adaptive resilience under continuous extensive management. Intensively housed sheep displayed lower overall cortisol (4.85 ± 0.20 pg/mg), yet summer heat stress revealed concealed welfare costs of confinement. Lambs showed lower cortisol levels than adult ewes; however, these differences were not statistically significant ($P > 0.05$). These findings support wool cortisol as a sensitive longitudinal welfare indicator integrating climatic exposure, housing conditions, and mobility-related stress. Beyond detecting acute responses, this biomarker captures cumulative physiological load and recovery dynamics, offering practical value for welfare-oriented management. Wool cortisol provides a scalable tool for monitoring welfare trajectories and identifying critical intervention periods, particularly during seasonal transitions in pastoral systems

Telomere Length as a Genomic Biomarker of Environmental Stress in Dairy Goats

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The identification of sensitive molecular indicators capable of capturing physiological responses to environmental stressors is a priority in livestock farming. In this context, molecular biomarkers can support precision livestock farming approaches by complementing sensor-based data with biologically meaningful information. Among these, telomere length (TL), a marker of cellular aging and oxidative stress, is emerging as a promising genomic biomarker in domestic species, being relatively rapid and cost-effective to measure under standardized conditions. Longer telomeres are generally associated with better health status and improved welfare. Moreover, TL dynamics are generally coordinated across tissues and reflect systemic physiological conditions, supporting their assessment in minimally invasive matrices such as milk. This study evaluated TL dynamics in dairy goats exposed to acute environmental stress under field conditions. Twenty grazing goats were monitored across three sampling points. A period characterized by high humidity and severe storm events was associated with a significant increase in serum cortisol concentrations ($P < 0.01$), accompanied by a significant decrease in milk-derived TL (expressed as T/S ratio; $P < 0.05$). Following the return to stable environmental conditions, both cortisol levels and TL values showed recovery trends. Correlation analysis revealed significant negative associations between TL and humidity ($r = –0.62$; $P < 0.01$) as well as dew point ($r = –0.58$; $P < 0.05$), supporting the sensitivity of TL to climatic stressors. These findings indicate that acute environmental challenges can induce measurable telomere shortening, reflecting increased cellular strain. Overall, TL responded consistently to short-term physiological stress and aligned with endocrine changes, supporting its role as an integrative biomarker of animal welfare. The integration of TL into field-based assessments may provide a valuable tool to detect early biological responses to environmental challenges, contributing to more informed and adaptive management strategies in small ruminant production systems.

Mapping Sleep Architecture in Nellore Beef Cattle: Validation of a Custom Polysomnography System

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Sleep is essential for mammals, and deprivation can impair immune function, increase energy expenditure, and heighten pain sensitivity (Bergmann et al., 1989; Moldofsky, 2001; Bonnet, 2005). Despite its importance, sleep in beef cattle remains poorly characterized. Most available data derive from lactating dairy cows, in which the physiological demands of lactation may influence sleep patterns. Therefore, this study aimed to validate a custom-made polysomnography (PSG) system to characterize sleep stages in Nellore beef cattle through electroencephalography (EEG), electromyography (EMG), and heart rate monitoring. Two Nellore steers (645 kg body weight) were fitted with a seven-channel PSG unit and monitored for 12 h (18:00 to 06:00). Four EEG electrodes were positioned on the frontal region to record cortical activity, while three additional channels recorded heart rate (bilaterally below the rib cage) and muscle tone via EMG (neck region). Animals were housed in 6.0 × 3.0 m pens without artificial lighting and fed a diet of corn silage and mineral supplements. During the recording period, two trained observers continuously monitored behavioral activity (idle, eating, ruminating), posture (standing, lying, tucked), and eye status (open or closed). Data were analyzed in 30-s epochs. Sleep stages were classified based on EEG, EMG, and heart rate patterns. Wakefulness was characterized by desynchronized, low-amplitude, high-frequency EEG activity (14–30 Hz), high muscle tone, and variable heart rate. Drowsing showed mixed synchronized/desynchronized EEG patterns with low-to-moderate amplitude and frequencies between 4 and 12 Hz, moderate muscle tone with maintained posture, and slightly reduced heart rate. Non-rapid eye movement (NREM) sleep was defined by synchronized slow-wave activity with high amplitude and low frequency (10–15 Hz), reduced muscle tone, and lower heart rate. Rapid eye movement (REM) sleep presented desynchronized, low-amplitude EEG activity (4–8 Hz), near-complete muscle atonia, and a less regular heart rate. Behavioral observations supported PSG classification, particularly to account for EEG artifacts caused by rumination. During the 12-h period, cattle spent 505 ± 26.87 min awake, 165.25 ± 68.24 min in drowsing, 25.25 ± 10.25 min in NREM sleep, and 24.5 ± 31.11 min in REM sleep. Within total sleep time, drowsing predominated (76.9%), followed by NREM (11.7%) and REM (11.4%). Inter-animal variability was greater than expected, suggesting that reactivity to the mounted PSG equipment may have influenced sleep expression. Compared with lactating dairy cows reported by Kull et al. (2019), which spent 87.37% of sleep time in drowsing, 11.58% in NREM, and 1.05% in REM, Nellore steers exhibited a proportionally greater REM duration, indicating possible physiological or production-system differences in sleep architecture. However, further investigation is required, as sleep data in dairy cows were collected over a 24-hour recording period. In conclusion, the custom-made PSG system reliably characterized brain activity and sleep stages in Nellore beef cattle. These findings establish a methodological foundation for investigating sleep quality as an innovative and objective indicator of welfare in beef production systems.

Social touch as a positive welfare phenotype in livestock: a cross-disciplinary synthesis of sensory and behavioral evidence

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Social touch is the most direct form of social contact, yet it remains comparatively understudied in behavioral and sensory research. Work in humans and rodents has focused on a specialized class of unmyelinated afferents, C-low threshold mechanoreceptors, as key mediators of affective touch. These skin fibers terminate as longitudinal lanceolate endings around hair follicles and are optimally tuned to slow, stroking at skin temperature. Their activation encodes hedonic aspects of tactile input and contributes to stress regulation and reward processing. Although C-low threshold mechanoreceptors have been identified, their peripheral organization has rarely been examined in relation to naturally occurring tactile behavior. By synthesizing findings from sensory neuroscience, behavioral biology, and animal welfare research, this review integrates mechanistic knowledge of peripheral tactile systems with empirical observations of social behavior in farmed animals. It highlights how stimulation of C-low threshold mechanoreceptors engages neural circuits linked to reward and stress regulation, and how these interactions can be conceptualized as measurable phenotypes. The review frames social touch as a biologically grounded phenotype, linking the tactile architecture to ethological observations and potential practical applications for livestock species. Neuroanatomical studies in rodents show that C-low threshold mechanoreceptors are distributed non uniformly across the body surface, with higher densities in regions such as shoulders and back. Behavioral observations indicate that these areas are preferentially targeted during affiliative interactions, suggesting a functional relationship between receptor topology and social touch. Comparable patterns have been reported in other mammals, and stimulation of receptor rich regions directly engages neural circuits involved in reward and stress regulation. Together, these findings indicate that tactile input from different body areas carries distinct biological significance. Methodological advances, including computer vision, allow quantification of spatiotemporal patterns of contact in group housed animals. Integrating these tools with knowledge about peripheral sensory organization could provide objective, low bias indicators of positive welfare which are urgently needed in livestock production. While the study of positive animal welfare has advanced, robust empirical indicators remain limited. Many current measures rely on context-dependent behaviors or subjective interpretations and have yet to be linked to mechanistic processes constraining reproducibility across species and systems. Social touch offers a promising avenue for addressing this gap. In livestock, interactions are often described broadly without detailed sensory analysis. Integrating social touch with sensory neurobiology provides a mechanistic scaffold, allowing spatiotemporal patterns of contact to be conceptually framed as a biologically grounded candidate phenotype of positive welfare, offering a testable approach for future research. Based on cross-disciplinary evidence, this review argues that social touch offers an interesting avenue in the search for biologically grounded and ethically relevant phenotype in livestock species. Situating tactile experience within the framework of positive welfare highlights its potential role in shaping stress regulation, social stability, and developmental trajectories. This approach contributes to a more comprehensive understanding of animal wellbeing, supports evidence-based refinement of management practices, and aligns livestock research with broader societal expectations regarding the promotion of positive states in farmed animals.

Benefits of temporary access to enriched environments for finisher pigs

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In recent years, in addition to minimising negative experiences, there is growing appreciation of the importance of positive experiences when it comes to ensuring good animal welfare standards. Pigs in conventional systems are often kept in pens offering limited opportunity to express natural behaviours. Economic limitations restrict pig producers' capability to modify these pens to provide outlets for rooting and exploratory behaviour. However, temporary access to an enriched pen could help to satisfy the intrinsic behavioural needs of pigs, without requiring structural modifications to the home pen. In this 2×2 factorial study, half of the pens across two housing systems – the "Conventional" vs "Alternative" (n=18 pens respectively) – were given access to an enriched pen (EA) for one hour, twice a week, for eight consecutive weeks during the finisher period. The remaining pens had no access (NA). Conventional pens were indoors and fully slatted (0.90m2/pig). Alternative pens had a higher space allowance (1.13m2/pig), 79% solid flooring, an internal resting area, and a covered 'outdoor' area where one wall was open to the outdoors. All home pens were equipped with a rack containing straw. Prior to each enrichment access session (EAS), EA pigs were played an audio cue for two minutes. Displays of anticipation (pushing the gate or other pigs to access the gate, head tosses, play) was observed continuously before the final EAS (Week 8, session 2) for all pens. Behaviour was observed throughout an entire EAS during the 3rd, and the final (8th) week of access, via six one-minute continuous observation sessions every ten minutes (6 minutes in total per pen). All counts of play-like behaviours (pivots, head tosses, flops, play fighting) were recorded. Tail (0 = no lesion, 1 = bite marks, 2 = open wound, 3 = infected wound), ear (0 = no lesion, 1 = superficial punctures, 2 = brown/red scab, 3 = bloody lesion on intact ear, 4 = bloody lesion on ear with missing tissue), and body lesions (0 = no lesion, 1 = brown scab, 2 = single red scratch, 3 = >1 red scratches, 4 = single lesion with broken skin, 5 = >1 lesion with broken skin) were scored on focal pigs (Conventional: n=4/pen; Alternative: n=10/pen) every second week. Data were analysed using SAS v9.4. Pigs in EA pens performed more anticipatory behaviours when presented with the audio cue ($P<0.05$) than pigs in NA pens when played the same audio cue. EA pigs performed more pivots and head tosses ($P<0.05$) during the beginning of the EAS. Thereafter the performance of these behaviours decreased and plateaued. Lesion scores did not differ between treatments. No differences were observed between housing systems for any of the measures taken. The performance of anticipation behaviours by EA pigs indicates that they considered EAS as a positive experience. The performance and plateauing of positive behaviours during EAS support this and signifies that one hour was sufficient for a complete behavioural outlet. Similarities in lesions across treatments demonstrates that returning to the home pens did not cause frustration in the pigs. This enrichment strategy is unlikely practical, but it helped us to understand pigs need for positive experiences in commercial production environments.

Damaging and play behaviours linked to brain neurochemicals in pigs

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Immune activation and stress can alter several metabolic pathways, thus affecting animal behaviour and emotional (affective) states. The aim of this study was to explore the relationships between positive and negative behaviours of pigs that may reflect their affective states and several brain analytes, using data from a previous experiment. From weaning (at 4 weeks) onwards, 46 female, pair-housed pigs were kept under high (HSC) or low (LSC) sanitary conditions and were offered either a diet deficient in the first 8 essential amino acids (NFC) or a choice between the deficient diet and a diet enriched with the same 8 amino acids (FC). Treatments HSC-FC and LSC-FC had 8 pens, whereas HSC-NFC and LSC-NFC had 4 pens. Durations and frequencies of damaging behaviour (biting pen mates' ears, tail, or other body parts), aggression, and play were recorded continuously on day 16 from 07:00 to 19:00h. On day 18, pigs were euthanised to obtain samples from the hippocampus and prefrontal cortex for analysis of concentrations of compounds involved in the kynurenic and serotonin (5-HT) pathways, and the catecholaminergic system. After transformation, residuals of the variables were extracted from a general linear model to remove treatment effects and allow examination across treatments. For each brain region, a principal component (PC) analysis was run to reduce the number of variables (neurochemical residuals). Pearson correlation coefficients (r) were calculated to assess the relationships between behavioural residuals and retained PCs; individual neurochemical residuals that did not load on any PC; and residuals of neurochemical ratios. To control for multiple comparisons, Storey's q-value procedure ($q < 0.10$) was applied. As results were similar for behavioural durations and frequencies, only the latter are reported. In the hippocampus, play fight ($r = 0.43$, $q = 0.02$) and total play ($r = 0.28$, $q = 0.06$) correlated positively with a PC with high loadings of 5-HT, dopamine, their respective metabolites 5-hydroxyindoleacetic acid and homovanillic acid, and norepinephrine (NE); and negatively (play fight: $r = -0.47$, $q = 0.01$; total play: $r = -0.33$, $q = 0.07$) with a PC with high loadings of tryptophan (TRP), kynurenine (KYN), and epinephrine (EPI). In the same brain region, play fight ($r = 0.62$, $q < 0.001$) and total play ($r = 0.32$, $q = 0.08$) correlated positively with the 5-HT/TRP ratio and negatively (play fight: $r = -0.61$, $q = 0.002$; total play: $r = -0.50$, $q = 0.007$) with the EPI/NE ratio. Hippocampal kynurenic acid (KA) correlated positively ($r = 0.42$, $q = 0.007$) with total play and negatively ($r = -0.27$, $q = 0.06$) with damaging behaviour. Similarly, the KA/KYN ratio correlated negatively with damaging behaviour ($r = -0.35$, $q = 0.05$) and positively with total play ($r = 0.42$, $q = 0.04$) in the hippocampus, and with total play in the prefrontal cortex ($r = 0.47$, $q = 0.03$). Lastly, damaging behaviour correlated positively ($r = 0.42$, $q = 0.06$) with the 5-HT/TRP ratio in the prefrontal cortex. In conclusion, our results point to a beneficial link between pig behaviour and KA, which has neuroprotective properties, and a negative link with the inflammatory pathway. The exploratory nature of the study precludes causal inferences, but these findings can form the basis for future research aimed at improving pig welfare and health.

Group and individual patterns in calf activity during a play arena test

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Calves' motivation to perform locomotor play, a putative indicator of positive welfare, can be assessed by releasing them into a large arena. Understanding how calves behave during such arena tests is essential to methodological design and correct data interpretation. While large individual variation in locomotor play has been mentioned by previous studies, baseline behavioural patterns at group and individual level during arena tests have scarcely been reported. This pilot study quantified and visualised group- and calf-level activity during a 20-min individual arena test (6 x 8 m, 30 cm-deep sand flooring) on two consecutive days. Calves (n = 12, 6-7 weeks old, individually housed) were fitted with a tri-axial accelerometer (HOBO pendant G data logger, Onset Computer Corp) on the left hind leg, which sampled at 4 Hz. For every 5 s of the arena test, the proportion of calves with at least one acceleration value < -2 or > 1.2 g was calculated, indicating high locomotor activity (hereafter referred to as 'playing'). At group level, calves played throughout the test, with activity being highest during the early stages. On d1, ~40 % of calves played immediately upon test start, with this peaking at ~60% around 4 min and then gradually declining to ~20% after 13 min. On d2, ~60% of calves played immediately upon test start, with this gradually declining to ~20% of calves after 7 min. At calf level, individuals played during (mean ± SD) 36.1 ± 10% of 5-s periods on d1 and 24.4 ± 6.3% on d2, ranging from 12.9% to 47.9 % on d1 and 10.8% to 31.2 % on d2. Visually, the temporal patterns varied, with some calves playing immediately after entering the arena but stopping quickly, some playing throughout, and some playing mainly mid-test. For example, on d1 the least and most active calves played during 6.7% vs. 75.0% of 5 s-periods in the first 5 min and during 3.3% vs. 48.3% of periods in the last 5 min. These results suggest that locomotor play was generally highest at the start of exposure, particularly on a second day, but could persist for up to at least 20 min. Calves differed in both the amount and timing of play. These findings can help determine appropriate play arena test durations depending on the research aim, and demonstrate that large individual variation should be taken into account regardless of test design. This work was co-funded by the European Union's Horizon Europe Project 101136346 European Partnership on Animal Health and Welfare as part of an internal project (SOA13).

Herd lying concordance: a novel measure of resting coherence in dairy cows

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Behavioural synchrony in social animals is considered a welfare-relevant indicator of social cohesion. In dairy cattle, lying behaviour constitutes a major portion of daily activity budgets, with long lying bouts (typically 60–99 min indoors) expected under favourable conditions. Conventional metrics classify intervals as synchronous when an arbitrary proportion of the herd is lying within an interval (e.g., >70%). Although practical, this dichotomous approach does not reflect the graded nature of collective resting. We introduce and evaluate a suite of continuous Herd Lying Concordance (HLC) metrics that quantify synchrony as the degree of alignment in individual lying time within a group. Using 15-min summaries of individual lying time, synchrony is expressed as a dispersion-based concordance index, where lower variation among cows indicates stronger behavioural alignment. This formulation captures partial overlap, avoids threshold dependence, and reflects the expectation that biologically meaningful resting occurs as sustained and coherent group-level patterns. Forty-two healthy, lactating Danish Holstein cows were equipped with leg-attached accelerometers (IDS i-QUBE, Peacock Technologies, UK) and allocated to 3 treatments (indoor loose housing, N=14; continuous grazing (CG), N=13; rotational grazing (RG), N=15) in which they remained for 12 weeks under spring–summer conditions. For each 15-min interval, we computed four dispersion-based HLC metrics: standard deviation (SD), mean absolute deviation (MAD), interquartile range (IQR), and Shannon entropy of the lying time distribution. The coefficient of variation was excluded from formal modelling due to lack of boundedness. Each computed HLC metric ranged from 0 (no synchrony) to 1 (almost perfect synchrony). We evaluated HLC metrics using GLMMs, including treatment, week, and their interaction as fixed effects, z-standardised weather covariates (relative humidity, temperature, precipitation), and a random intercept for treatment group. The HLC metrics were evaluated based on model fit (AIC), treatment effect detectability (i.e., signal-to-noise ratio defined as mean absolute treatment contrast divided by its standard error), temporal stability (mean SD of weekly values across treatments), and robustness to outliers (ratio of the SD to the MAD). A robust HLC metric combines lower AIC, higher signal-to-noise ratio, higher temporal stability, and lower outlier sensitivity. Validation also addressed construct validity against 70% threshold synchrony (sync70) and discriminant validity against daily lying time and lying bout duration. The MAD-based formulation showed the best model fit (−1365.2), highest temporal stability (0.015), signal-to-noise ratio of 13.7, and high outlier robustness (1.108). Construct validity was confirmed via Wilcoxon rank-sum test (P<0.001) and logistic regression. Discriminant validity was supported by a negative correlation with lying time (rs=−0.55; P<0.001) and a positive correlation with mean lying bout duration (rs=0.52; P<0.001), confirming HLC-MAD captures resting coherence independently of duration. Night-window stability (22:00-04:00) was 3–6× greater than sync70. Incremental validity was confirmed by model comparison (ΔAIC=66.2 over sync70 alone). Both grazing treatments showed higher HLC-MAD than indoor housing (CG=0.964 and RG=0.966 vs. Indoor=0.924; P<0.001) and did not differ from each other (P>0.1), suggesting the benefit is attributable to pasture access per se. HLC-MAD provides a biologically grounded, threshold-free indicator of collective resting synchrony in dairy cattle.

Effect of a two-hour daily exit on milk quality, activities and outside behavior of Italian Simmental lactating cows

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Mixed management systems combine indoor confinement plus an outdoor time for cows to pasture or to use as an exercise area. They had arisen as a new trend in dairy cows' management mostly due to welfare concerns from consumers. But, raising cows in a free stall with an automatic milking system while offering a daily exit in a paddock may be challenging for farmers. This study investigated the effect of a two-hour daily exit on milk traits, activities and outside behavior of dairy cows. A four-week trial was conducted using 10 lactating cows divided into two homogeneous groups (IN and OUT) for DIM (129.2 ± 18.8), parity (3.0 ± 1.4) and absence of mastitis and lameness. The experimental design was a crossover (2 groups x 2 periods), so each group was alternatively IN or OUT in each period. Both groups were reared in a free-stall, but meanwhile the IN group remained always inside, the OUT group had a two-hour daily exit (15:00-17:00) to a paddock. Milk yield, milk quality, and activities were monitored via individual collars linked to the automatic milking machine (AMS). Activities included the number of milkings, feeding (min/d), feed intake (kg/d), and rumination (min/d, min/h). Outdoor behaviors were monitored using six digital video cameras covering the paddock area (1500 m2) and connected to a digital video recorder. Recordings were analyzed by a trained observer. Climate data (relative humidity, temperature, wind speed, solar radiation, and rainfall) and the adjusted Temperature-Humidity Index were used to better understand outside behaviors. Statistical analysis followed a mixed model. Results showed a higher daily number of milkings for the IN group (3.02 vs. 2.88; $P<0.05$), but no differences in milk production (on average 32.6 kg/d) or milk quality traits such as: fat, lactose, and SCS. Milk protein was higher for the IN group (4.81 vs. 4.80 %; $P<0.05$). Eating behavior parameters were not affected by the treatment with a daily average of 236 minutes of feeding and 4.53 kg of compound feed intake. Daily rumination was higher for the IN group (485 vs. 472 min), primarily due to the lower rumination of the OUT group during the exit (at 16:00) (19 vs. 7 min). The main outdoor behaviors were standing (60.45%), grazing (38.10%) and ruminating (11.81%). Rainfall affected ($P<0.05$) walking, standing, positive interaction, playing and sexual behavior. In conclusion, offering lactating dairy cows a two-hour daily exit in the afternoon appears to be an effective mixed management system, allowing cows to exhibit natural behavior while maintaining milk production and composition comparable to a full indoor system. This system could be viable for Italian farmers to add value to the final product but the provision of some shade inside the paddock should be considered in order to avoid discomfort in cows. Acknowledgement: Funded by the Regional Agency for Rural Development (ERSA) and the European Union's Horizon Europe Project 101136346 EUPAHW: SOA13, "Positive animal welfare".

Technologies for on-farm measurement of positive animal welfare: A scoping review

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Positive animal welfare (PAW) goes beyond the current focus on minimizing negative states (such as pain, fear, injury, and disease) by emphasizing the inclusion of positive experiences and emotional states in animals. This study was part of the COST Action LIFT (CA21124), and aimed to systematically identify and characterize technologies that have potential to be used on commercial farms to monitor aspects of PAW in livestock. A scoping review was conducted in July 2024 using PubMed, Scopus and Web of Science. Eligible studies were original research written in English and published between 2014 and 2024. Five technology-specific search queries were used, targeting automated behaviour monitoring, wearable sensors, sound-based technologies, imaging, and integrated data-driven systems. After duplicate studies removal, 4652 papers were screened based on their abstracts by 33 assessors using predefined inclusion criteria. A total of 758 papers were retained for data extraction. The percentage of papers for each animal category were as follows: cattle (67%), pigs (12.8%), poultry (8.6%), and small ruminants (8.6%). The most common measured indicators were body posture/movement (25.89%), rumination (24.1%), and behavioural synchrony (8.67%), while all other indicators individually accounted for less than 8% of the studies. Hardware-based technologies predominated (82.5%), in which the validation relied mainly on internal procedures (35.6%) or human observations (52%) as a gold standard. Around half of the identified technologies were considered applicable under commercial farm conditions by the assessors. Overall, the results indicate that some PAW indicators can be assessed with the help of available technology. However, most of the measurements assessed general welfare or behaviour, not specifically PAW. Defining measurable PAW indicators is needed to develop practical methodologies and technological systems. Furthermore, wider application across livestock species and stronger external validation is required to support robust on-farm use and provide reliable outputs.

Identifying animal-based indicators for positive animal welfare in laying hens

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Animal welfare can be defined as the experience of life viewed by the animal itself. Factors that affect welfare are those which create positive or negative mental states. The focus in animal welfare science has accordingly shifted from merely minimising suffering to also promoting positive experiences and a good life. It has even been suggested that positive experiences and their benefits are crucial to animal welfare. Following, there is an interest in objectively measuring positive animal welfare (PAW). However, feasible indicators for measuring PAW on farm are still needed. The aim of this study was therefore to identify behavioural indicators related to positive experiences in brown laying hens and it was hypothesized that hens would show different types, duration or sequence of behaviour during and/or after receiving a highly valued resource than control animals. To test the hypothesis, an experiment was carried out with 12 laying hens receiving mealworms to evoke a positive mental state and 12 control hens not receiving a resource. The experimental arena consisted of a front and back area divided by a door. The hens were placed in the front part of the pen, the door was opened after 10 seconds and hens were able to move to the back area where they had access to the resource. All hens were tested three times in pairs, while receiving a different resource each time 1.) freely available mealworms, 2.) mealworms in a snack ball or 3.) mealworms in litter. Test and control hens remained in the arena for a total of 20 minutes. Control hens did have access to the back area, but did not receive the different resources. All laying hens were trained before testing began to ensure habituation to the arena. Additionally, test hens passed the training when showing anticipatory behaviour and ability to move through the door. Behavioural observations at hen level were executed continuously from video recordings. Furthermore, tail movements and position were scored. Currently, the videos are being analysed and this data will be statistically analysed afterwards. Pre-liminary results indicate that the tail movements and tail position are linked to availability of the resource and differ per type of resource. Furthermore, duration and frequency of behaviour seems to differ per resource. Future research should focus on testing validity of found indicators on farm. The finalized results will be presented during the EAAP conference. This work was co-funded by the European Union's Horizon Europe Project 101136346 European Partnership on Animal Health and Welfare as part of an internal project (SOA13).

Sacred Care and Scientific Compassion: Integrating Positive Animal Welfare into Lifelong Management Systems of Indian Gaushalas

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Gaushalas, or cow shelters, have been around for a significant time and have been integrated into the fabric of Hindu religious and ethical philosophies. The Government of India report from 2023 provides a state-by-state breakdown of gaushala density. The highest density can be seen in the northwest and central parts of India: Rajasthan has 2,269 gaushalas, Gujarat has 1,418, Madhya Pradesh has 905, Haryana has 640, and Uttar Pradesh has 582. A slightly lower density can be seen in Punjab with 447, Maharashtra with 222, Karnataka with 220, Himachal Pradesh with 221, Telangana with 175, and Chhattisgarh with 117. Other areas have fewer than 40 or no gaushalas at all. This corresponds with how cattle demographics, religious acceptance, and the demands of caring for old or unproductive animals intersect.Unlike dairy farming, where there is a clear end product with a limited time frame before it is sold or consumed, gaushalas have animals in their care for a much longer time. The focus then changes from the end product to the overall life experience. However, research has shown that there are problems with gaushala management. The common approach is protective and follows the 1960 Prevention of Cruelty to Animals Act. It seeks to ensure survival, provide sufficient food, and prevent slaughter. However, welfare science now suggests that simply not being in pain is not necessarily indicative of good welfare. The Five Domains approach provides a clear structure for welfare assessment. It covers nutrition, environment, health, behavior, and mental state. It can be used to assess positive welfare as well as negative welfare. In gaushala situations, small imbalances in nutrition can have a significant impact over time.This study offers a framework for a norm-based, management-focused approach to integrating PAW into gaushalas. From a farm management perspective, three areas for greatest leverage are: firstly, improving nutrition beyond mere maintenance through forage variety and rumen health; secondly, re-designing the physical space to increase space complexity, provide adequate shade, and support stable social groups; and lastly, adding behavioral activities and providing choices to increase animal agency and positive emotions. Even marginal daily improvements, when multiplied over 3,650–7,300 days of post-productive life, may substantially alter cumulative welfare outcomes.Gaushalas are seen to be hybrid institutions that combine elements of religious obligation or duty (dharma), non-violence or ahiṃsā, and civil society engagement with large-scale herd management. Their ethical legitimacy is strong, though welfare outcomes are variable. Once we think of shelters as dynamic management systems rather than static sanctuaries, the transition from mere protective welfare to positive welfare becomes practically possible. The study's findings suggest that integrating PAW into life-long cattle management is not simply an ethical addition; it is a structural imperative to guarantee that extended life translates into a life worth living.

Effect of enrichment provision and pair-housing on the development of calf locomotor play during the pre-weaning period

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Play is an easily recognisable, non-invasive measure of an animal's current affective state, which typically decreases when animals experience threats to welfare (e.g. hunger or pain). However, compared to welfare threats, an empirical link between positive emotions and increased play behaviour in non-human animals is less well established. Therefore, to evaluate the validity of play behaviour as a positive welfare indicator in calves (Bos taurus), we investigated how play changes over the first weeks of life based on whether or not calves were kept in preferred housing conditions. 42 calves in seven blocks of six were housed generally within 24 hrs of birth in individual (7.5x3m) or pair (7.5×4.5m) pens under enriched (with a swinging non-motorised brush and hay net) or control conditions in a 2×2 factorial design (each block featured one pen from each of the four treatments). At weeks 2, 3, 4, 5 and 6 following introduction of the final calf into a given block, 28 calves (one focal calf per pen) were equipped with hind leg-mounted accelerometers (HOBO pendant G data loggers) sampling at 1Hz on the vertical (x-) axis from 05:00-23:00 for two consecutive days (two 18 hr periods). Currently, completeness and quality of accelerometer data records have been verified, and relevant acceleration features calculated (i.e., peak counts at different thresholds, means of highest and lowest values, variance, and several features capturing change between consecutive measurements) at 10 s windows. These features will be compared to play observations coded from video footage taken at week 6 into the experiment, to develop and test an algorithm that can validly detect calf play (one-zero sampling for each 10 s window). From here, we will establish whether this algorithm can also be applied to identify play in calves of younger age cohorts, using video observations taken at week 2. Finally, the validated algorithm will be applied to the full data set (i.e. all calves and age cohorts) to study/compare differences in locomotor play development over the pre-weaning period between our four housing treatments. We predict that calves able to access resources that they prefer and value (enrichment items and companions) will be in a more positive mental state and therefore express higher levels of locomotor play compared to calves in less preferred housing situations. Our findings will be ready to present in September at the EAAP conference. Our research was co-funded by the European Union's Horizon Europe Project 101136346: European Partnership on Animal Health and Welfare (EUP AH&W) as part of SOA13: Assessment of positive welfare defining animal-based measures.

Black soldier fly larvae in poultry feed: nutrition, functionality, and challenges for sustainable production in a circular economy

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Insects constitute a promising source of animal-derived protein, fat, and minerals for poultry nutrition. Among the EU-approved insect species, black soldier fly larvae (BSFL) are of particular interest due to their comparatively high nutrient and energy conversion efficiency as well as their remarkable flexibility regarding rearing substrates. In addition, functional properties such as antimicrobial peptides, lauric acid, chitin, and mineral accumulation, together with their potential use as edible environmental enrichment for poultry, further enhance the appeal of BSFL as feed ingredient. BSFL-derived products can at least partially replace conventional protein sources without compromising growth performance, feed efficiency, or product quality in both broilers and laying hens. However, optimal inclusion levels of BSFL in poultry diets remain under discussion, as studies have used non-standardized BSFL products (e.g., defatted vs full-fat meal, dried or live larvae) with variable protein and fat contents, which influence amino acid and energy availability, digestibility, and consequently animal performance. Beyond their nutritional value, BSFL contribute to circular bioeconomy strategies through efficient biomass conversion and reduced reliance on resource-intensive feed ingredients. Nevertheless, the economic and ecological sustainability of BSFL production as poultry feed strongly depends on the availability of substrates that have little or no potential for direct use in food or feed production. From a systems perspective, BSFL should exhibit substantially higher nutrient and energy conversion efficiencies than poultry to justify their production on high-quality substrates. This limitation arises from the so-called double transformation of nutrients and energy, whereby additional nutrient and energy losses occur during insect production prior to their use as livestock feed. The production of BSFL as poultry feed involves a double biological conversion, and the associated trophic losses highlight the need to rely primarily on substrates with low or no value for direct human or livestock consumption. Consequently, nutrients for BSFL should primarily originate from biomass that is inedible to humans and livestock species. This prioritization of biomass use underscores the need for innovative technologies to exploit sustainable substrate sources for BSFL production. The first aim of this work is to provide an overview of the nutritional quality of BSFL protein, fat, and minerals, and to evaluate nutritional, physiological, health, and behavioural responses of poultry to dietary intake of BSFL. Second, challenges related to sustainable BSFL production on low-quality substrates, which are upgraded through technological approaches (e.g., fermentation and other pre-treatments) are discussed. In this context, ongoing activities within the newly launched EU-wide project NUTRIFEEDS aimed at developing sustainable BSFL-based feeding strategies for poultry will be presented.

Dietary inclusion of Hermetia illucens oil in dairy cows: effects on rumen microbiota and fermentation parameters

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Including lipids in the diet of dairy cows is a common practice aimed at increasing the dietary energy density while also modulating rumen microbiota to reduce CH4 emissions. However, traditional lipid sources are often criticized for their high environmental footprint, highlighting the need for more sustainable alternatives. This study aimed to assess whether the oil extracted from Hermetia illucens (HIO) larvae, which has a potentially lower footprint, could serve as a viable alternative to fractionated palm oil (FPO) in dairy cow nutrition without causing negative effects on rumen fermentation parameters and microbiota in dairy cows. A 50-day trial was conducted with two equivalent groups of thirteen lactating Valdostana Red Pied cows each. The two groups were fed a diet characterized by a 70:30 forage:concentrate ratio, which differed only for the concentrate lipid source, being either HIO or FPO (0.9% of the diet DM). Individual rumen fluid samples were collected on the last day of the trial. In vivo analysis included microbiota composition (DNA sequencing), and protozoal counts. Rumen fluids were also used as inocula for in vitro incubations (24 h) to determine total gas and CH4 production, pH, ammonia and volatile fatty acids. Statistical analysis was performed using the MIXED procedure of SAS software ($P<0.05$). Spearman correlation evaluated associations between bacterial genera and fermentation profile. No differences in the tested fermentation parameters were observed, with the only exception being butyrate production, higher with the HIO diet compared to the FPO diet (16.9 vs. 12.0 mol/100 mol, respectively; $P<0.001$). This higher production was concomitant with a greater abundance of large Entodiniomorphs protozoa (9.14×103 cells/mL vs. 1.17×103 cells/mL in the HIO group and FPO group, respectively; $P=0.001$), which are known to directly contribute to butyrate production. Interestingly, C12:0, the main individual fatty acid in HIO, is known for its antimicrobial properties and therefore a protozoal reduction was expected. However, literature suggests this effect being dose dependent. Consistent with a higher abundance of protozoa, an enrichment in other archaeal or bacteria genera typically associated with them, such as Methanobrevibacter ($P<0.01$) and Treponema ($P<0.05$), was observed in rumen fluid of HIO-fed animals. To add, several fiber-degrading Firmicutes, including Pseudobutyrivibrio, were less abundant in the HIO group ($P<0.05$). No differences in α- or β-diversity were observed between the two groups ($P>0.05$). Spearman correlation analysis further revealed positive associations between C12:0 intake and Methanobrevibacter and Treponema abundance ($P<0.05$), suggesting that the C12:0 content of HIO promoted the observed microbial shifts. These findings highlight that a low dose of HIO only exerts modest effects on rumen fermentation parameters and microbiota shifts, suggesting the possible use of HIO in dairy cow diets as an innovative and alternative lipid source. Research supported by Hi-Ga project; Green Era Hub on Agri Food and Biotechnology.

Black soldier fly larvae (Hermetia illucens) fat as an alternative fat source to coconut fat in milk replacer for calves

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The increasing global demand for food and growing competition for natural resources necessitate the development of sustainable feed alternatives in livestock farming. Insect-derived ingredients have emerged as promising feed sources due to their efficient nutrient conversion and potential for circular bioeconomy applications. Within the European Union, processed animal proteins from insects were approved for poultry and pig diets, whereas insect protein remains restricted for ruminants' diet. However, insect-derived fats are permitted in calf nutrition. Black soldier fly larvae (BSFL, Hermetia illucens) represent a promising fat source due to their ability to efficiently convert organic waste into high-quality biomass and offer a local alternative to conventional fats such as palm and coconut fat. Compared to coconut fat, which is largely imported from tropical regions, BSFL fat can be produced regionally, reducing environmental impact while maintaining nutritional value. BSFL fat is characterized by a high proportion of saturated fatty acids, particularly lauric acid, resulting in a fatty acid profile similar to that of coconut fat, which is widely used in calf milk replacer (MR) formulations. While BSFL products have been successfully integrated into poultry, fish, and swine diets, their application in calf nutrition remains largely unexplored. This study evaluated the effect of feeding MR formulated with BSFL fat as an alternative fat source for calves. A total of 24 male Simmental calves (33.5 days of age; initial body weight = 76.0 kg) were allocated equally to two dietary treatments (n = 12 calves per treatment): CON = MR containing 50% sunflower oil and 50% coconut fat as the fat source; LAR = coconut fat completely replaced by BSFL fat. Both MR formulations were isoenergetic and isonitrogenous. Calves were fed for 63 days using an automatic MR and concentrate feeder equipped with transponders to allow individual intake recording. The feeding program consisted of five weeks of intensive MR feeding (maximum 12 L/calf/day), followed by four weeks of gradual weaning to 2 L/calf/day in the last week. Concentrate feed was available at 9.8 kg/calf/day, and water and hay were available ad libitum. Calves were housed in one group per treatment (12 calves per pen) in a naturally ventilated barn with straw bedding. Body weight (BW) and health parameters (e.g., fecal consistency score) were recorded weekly. Data were analyzed using one-way ANOVA ($p < 0.05$). The results of the study showed no significant difference in BW or average daily gain between the two dietary treatments. However, LAR-fed calves had a higher MR intake than CON, with significant differences observed at week 3, while concentrate feed intake was significantly lower in LAR calves than in CON calves at weeks 8 and 9. This resulted in a significantly reduced total dry matter intake and lower metabolizable energy intake in LAR calves. Feeding behavior showed no significant differences between diets for rewarded visits, unrewarded visits, or total visits, while drinking speed (mL/min) was significantly higher in LAR compared to CON. There was no significant effect of dietary treatment on fecal consistency score.

Influence of chitinase inclusion level and application method on the digestibility and utilization of black soldier fly meal in juvenile rainbow trout

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The inclusion of black soldier fly (BSF) meal in rainbow trout (Oncorhynchus mykiss) diets has been widely investigated, generally showing positive effects on growth performance. However, nutrient digestibility may vary depending on the type and processing of the insect meal. Therefore, strategies to enhance BSF meal utilization require further investigation. The present study aimed to evaluate the effects of different chitinase inclusion levels and enzyme application methods in juvenile rainbow trout diets containing BSF meal. A basal control diet including 10% BSF meal was formulated (C). Chitinase (Aspergillus niger) was directly incorporated into the diets at increasing inclusion levels of 0.25%, 0.50%, and 0.75% (CH1, CH2, and CH3, respectively). In addition, a pre-treated BSF meal subjected simultaneously to enzymatic digestion with 0.75% of chitinase and heat treatment was evaluated (PRO2). To distinguish the effects of thermal processing from enzymatic activity, a heat-treated BSF meal without chitinase was included as a separate treatment (PRO1). At the beginning of the trial, a total of 480 fish (16 g ± 0.13) were allocated to 24 tanks, which were then randomly assigned to the different dietary treatments. Fish were weighed every two weeks to monitor growth curve. The trial was ended when the fish had tripled their initial body weight. To determine diet digestibility, Celite was included in the feed as an inert marker at 1%. Feces were collected twice daily over a 7-hour period for 35 days by siphoning the tanks and subsequently filtering the recovered material. The evaluated parameters included survival rate (SR), final body weight (FBW), feed conversion ratio (FCR), and apparent digestibility coefficients of dry matter (ADCDM), crude protein (ADCCP), and gross energy (ADCGE). Data were analyzed using one-way ANOVA, followed by Tukey's post hoc test (SPSS v28.0; $p<0.05$). SR was not affected by the dietary treatments ($p>0.05$). However, FBW and FCR were significantly improved in the CH1 group compared to CH3 ($p<0.05$), while the other groups showed comparable results ($p>0.05$). ADCDM and ADCGE were not influenced by the experimental diets ($p>0.05$). In contrast, the ADCCP was significantly higher in the control group compared to all other treatments ($p=0.01$). Overall, chitinase supplementation did not produce consistent improvements in growth performance or nutrient digestibility compared to the control diet, while the moderate supplementation level showed slightly better growth performance than the highest level. The results indicate that 0.75% of chitinase inclusion may exert a negative influence on growth performance than 0.25% level, without providing digestibility benefits. The HiGa project is supported within the Green Era Hub call by the Green Development and Demonstration Programme, GUDP, under the Italian Ministry of Agriculture, Food Sovereignty and Forests (MASAF) (CUP: GASL_RIC_COMP_24_02).

Black soldier fly larvae (Hermetia illucens) meal as an alternative protein source in organic free-range laying hen production

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With the EU Organic Regulation (EU) 2018/848 requiring 100% organic feed in organic livestock production by 2027, the demand for high-quality, regionally produced, and organic protein sources in Germany is increasing. At the same time, organic egg production faces challenges due to the limited availability and environmental impact of conventional protein sources such as soybean or fish meal. Insects represent a promising alternative because of their high protein quality, efficient resource use, and potential for production within circular agricultural systems. This study investigated the effects of feeding black soldier fly larvae (BSFL) meal as a substitute for soybean in organic diets on production performance and egg quality of Lohmann Brown laying hens. A total of 160 18-week-old laying hens were randomly assigned to two dietary treatments (4 pens/treatment, 20 hens/pen, free-range system). The treatments consisted of an soy-based control diet (SOY) and a BSFL meal-based diet (LAR). Hens in the SOY group received a commercial organic diet containing 13.5% soy. In the LAR treatment, soy was completely replaced by BSFL meal. After a two-week adaptation, birds were fed for 14 weeks (20-34 weeks of age). Body weight was recorded at week 20, 25, and 34 weeks of age, and feed intake was measured weekly. Egg production was recorded daily to calculate weekly percentage laying performance. At the end of the experimental period, 10 eggs per pen (40 eggs per treatment) were collected for egg quality analysis. Data were analyzed using one-way ANOVA ($p<0.05$). Feeding BSFL meal in organic diets did not affect hens´ production performance. During the whole experimental period, no significant differences were observed between treatments in average daily feed intake (SOY=109 vs. LAR=110g/hen/day; $p>0.05$), percentage laying performance (SOY=90.9 vs LAR=89.7%; $p>0.05$), individual egg weight (SOY=57.8 vs. LAR=58.5g/egg; $p>0.05$), or feed conversion ratio (SOY=2.07 vs. LAR=2.11g feed/g egg mass; $p>0.05$). Egg quality analysis showed that eggs from LAR-fed hens were longer (SOY=54.7 vs. LAR=56.1mm; $p<0.01$), resulting in a significantly lower shape index (SOY=80.2 vs. LAR=78.4; $p<0.01$). However, LAR-fed hens produced numerically heavier eggs (SOY=59.6 vs. LAR=60.9g/egg; $p>0.05$), resulting in higher proportion of marketable egg weight classes M and L (SOY: 15% S, 63% M, 23% L, 0% XL vs. LAR: 0% S, 73% M, 25% L, 3% XL), which is of economic relevance. LAR-eggs showed a significantly lower albumen height, resulting in reduced Haugh units (SOY=87.4 vs. LAR=82.3, $p<0.01$). Moreover, LAR-eggs contained a significantly higher yolk proportion (SOY=25.7 vs. LAR=27.2%, $p<0.01$), which was associated with a tendency towards lower albumen (SOY=61.4 vs. LAR=60.3, $p<0.1$) and eggshell proportions (SOY=12.9 vs. LAR=12.5, $p<0.1$). Eggshell breaking strength and thickness were not affected by dietary treatment. However, yolk color, as measured by the DSM ROCHE color fan, was significantly lighter in LAR eggs compared to SOY (SOY=7.1 vs. LAR=6.3, $p<0.01$). In conclusion, replacing soy with BSFL meal represents a suitable and sustainable alternative protein source for laying hens in organic production systems, maintaining productive performance while inducing only minor changes in egg quality characteristics. The study was conducted as part of the EIP-agri project “LARF-Pick” and funded by the Ministry of Economics, Transport, Agriculture, and Viticulture, Rhineland-Palatinate, Germany.

Effects of three feeding strategies based on black soldier fly larvae on gut health and redox status in fast-growing chickens

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Black soldier fly (BSF) larvae are a promising alternative protein source for poultry, and have been reported to enhance gut health due to their richness in chitin, lauric acid, and antimicrobial peptides. However, in vivo studies have shown inconsistent health outcomes, likely due to differences in dietary inclusion levels and characteristics of the BSF material used. This study investigated the mechanisms by which three BSF materials affect the systemic and gut health of fast-growing chickens. Two hundred sixty-four day-old Ross 308 male chicks were assigned to one of four experimental diets until d35 (one pen per diet, each bird served as the experimental unit): a control diet (C); a diet with 10% of defatted larvae meal (LM), and two diets associating a deconcentrated pelleted feed with the provision of either live (LL) or dried (DL) BSF larvae, representing 15% of daily dry matter (DM) intake. Daily performance was measured individually using electronic feeders. At d35, 15 birds per diet were randomly selected for plasma, jejunal mucosa, caecal contents, and thigh muscle collection. Gut health was assessed through markers of (1) barrier integrity, absorptive, and immune functions, (2) redox status, and (3) microbiota composition. Jejunal histomorphometry was evaluated by H&E staining. Expression of gut-related genes in the jejunal mucosa was quantified by RT-qPCR. Plasma redox status was determined by measuring total antioxidant capacity and lipid peroxidation (TBARS). Caecal microbiota composition was assessed by sequencing the 16S rRNA V3/V4 region. Data were analysed using One-Way ANOVA followed by post-hoc tests. DL birds showed the lowest growth rate throughout the period ($P<0.001$; -10% compared to C). As a possible compensatory mechanism, these birds had a larger villus surface area ($P<0.01$; +32%<C). Upregulation of nutrient transporter genes (PAT1, SLC7A6, SLC1A1, and SLC5A) in DL birds, compared to C and LM, suggested an adaptive response to lower nutrient availability. However, the upregulation of NF-kB, TNFα, and EXFABP in the DL group ($P<0.05$) indicates a pro-inflammatory gut response. Increased expression of tight junction genes related to paracellular barrier functions (CLDN10, OCLN, TJP2, and MMP2) was also observed in the jejunal mucosa of DL birds ($P<0.05$), compared to C, potentially to counteract inflammation and maintain barrier function. At the systemic level, an increase in plasmatic and leg muscle lipid peroxidation was observed in LL birds compared to C and LM ones ($P<0.01$), with intermediate levels for DL ones, but total antioxidant status remained unchanged among diets. Caecal microbiota beta-diversity metrics were modified, with a significant increase in bacterial symbionts Bacteroides, Lactobacillus, and Ruminococcaceae UCG-005 in LM birds ($P<0.05$). In conclusion, 10% LM reshaped the caecal microbiota profile without impairing growth. In contrast, 15% BSF larvae, particularly dried, triggered a pro-inflammatory intestinal response and systemic oxidative stress, necessitating compensatory mechanisms in the gut. Further research is needed to identify the specific molecules driving these results to optimize BSF inclusion in poultry diets.

Does gut health have a key role in shaping growth performance in insect-fed broilers?

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Gut health is considered a key driver of growth performance in broiler chickens, but integrated analytical approaches encompassing gut microbiome, histomorphometry, and mucin histochemistry analyses for the identification of specific microbial, metabolic, and structural intestinal biomarkers associated with variations in bird productive performance are still very limited. The present study investigated the relationships among comprehensive gut health parameters and growth performance in broiler chickens fed diets containing Hermetia illucens (HI) and Tenebrio molitor (TM) meals at 5% or 10% inclusion levels, either as single ingredients or in a 1:1 mixture. A 37-day growth trial was conducted using 420 male Ross 308 broiler chicks randomly assigned to seven dietary treatments (6 replicates/diet, 10 birds/replicate): C (control), HI5 (5% HI), HI10 (10% HI), TM5 (5% TM), TM10 (10% TM), MIX5 (5% MIX), and MIX10 (10% MIX). An integrated analytical approach encompassing caecal microbiome characterization (amplicon sequencing, shotgun metagenomics and targeted metabolomics), and jejunal histomorphometry and mucin histochemistry was employed to identify microbial, metabolic, and structural intestinal biomarkers mechanistically explaining variations in bird productive performance. High-performing birds (MIX5 and TM5) exhibited caecal microbiomes enriched in short-chain fatty acids-producing taxa, including Veillonellaceae, Butyricicoccus, and Limosilactobacillus crispatus, which correlated positively ($P<0.05$) with feed conversion rato (FCR), average daily gain (ADG), and average daily feed intake (ADFI). These groups demonstrated favorable metabolomic shifts, including increased concentrations of dopamine, tyramine, malic acid, and orotic acid, alongside reduced levels of metabolic dysfunction markers such as 3-deoxyglucosone and hexanoylcarnitine ($P<0.05$). The preservation of gut mucin balance, particularly acidic sulphated mucins, was strongly associated with higher ADFI ($P<0.01$), improved FCR ($P<0.05$), and greater body weight ($P<0.05$). Conversely, low-performing birds (MIX10 and HI10) displayed gut microbiome signatures dominated by Ruminococcaceae, Alistipes, and L-Eubacterium, which are taxa negatively correlated with feed efficiency and associated with altered purine metabolism ($P<0.05$). These microbial shifts coincided with compromised gut morphology, including tendency towards reduced villus height in MIX10 birds ($P=0.070$) and decreased mucosal and muscular layers ($P<0.01$), which correlated positively with ADG ($P<0.05$) and negatively with FCR ($P<0.05$). The TM10 diet maintained stable performance despite high inclusion level, potentially due to beneficial taxa (Limosilactobacillus crispatus and Tyzzerella) and marked reduction in Campylobacter jejuni abundance (false discovery rate <0.05), alongside decreased antimicrobial resistance genes ($P<0.05$). Jejunal inflammation remained unaffected across treatments, indicating that growth modulation was primarily driven by microbiome–mucosal interactions rather than inflammatory processes. These results underscore the complexity of host–microbiome–diet interactions and identify specific microbial taxa (Butyricicoccus, Veillonellaceae and Limosilactobacillus crispatus), metabolites (dopamine and tyramine), and mucosal features (villus height, mucosal and muscular layers and acidic mucin composition) as potential biomarkers of optimal performance in broilers fed insect-based diets, suggesting that 5% inclusion levels optimize gut functionality and productive outcomes.

Effect of black soldier fly larvae oil and feed withdrawal on meat quality parameters in broilers subjected to cyclic heat stress during the final growth period

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Medium-chain fatty acids (MCFAs) are rapidly oxidized, less susceptible to lipid peroxidation, and provide readily available energy, which may help preserve oxidative stability in broiler muscle under heat stress (HS). In addition, controlled feed withdrawal (FW) during peak heat periods may reduce metabolic heat production, further protecting muscle from oxidative damage. It is hypothesized that the inclusion of black soldier fly larvae oil (BSFO) as a lipid source rich in MCFAs, together with a strategy of FW may attenuate HS induced deterioration of broiler meat quality. A total of 540 one-day-old Cobb 500 broilers were randomly allocated to 54 pens and fed the same commercial diets from 1 to 20 days of age. Broilers were then divided into 6 groups (9 replicates per treatment; 10 birds per pen) following a completely randomized design. Two experimental groups were kept under thermoneutral (TN) conditions and fed isoenergetic (3,079 kcal/kg) and isoproteic (19% CP) corn soybean diets supplemented with 5.5% soybean oil (SBO) or BSFO from 22 to 35 days of age. The other four groups were also housed under TN conditions until day 29, then exposed to cyclic HS (temperature-humidity index 80) for 6 hours daily from days 30 to 35. These birds received SBO or BSFO diets, and two of the groups additionally underwent a 6-hour FW during the peak HS period. Birds were euthanized using CO_2, and carcasses were chilled at 1 °C for 2 h. Breast samples were collected from 9 birds per treatment then stored at 4 °C under LED lighting to simulate commercial retail display conditions. Water-holding capacity (WHC) was determined at 72 h postmortem, whereas color stability and lipid oxidation measured by thiobarbituric acid reactive substances (TBARS) were evaluated during refrigerated storage (4 °C) at days 1, 4, and 7. Main effects stress (TN or cyclic HS), fat source (SBO or BSFO), feed withdrawal and storage time for color and TBARS and their interactionswere analysed with orthogonal contrasts using the Mixed procedure of SAS. WHC was significantly higher in meat from birds under TN conditions than under HS ($P < 0.001$), with no effect of oil type or FW. Meat lightness (L*) was also higher in TN conditions compared with HS ($P = 0.035$). Meat redness (a*) tended to increase in the BSFO groups subjected to FW under HS ($P = 0.059$), whereas yellowness (b*) was higher in treatments with FW, regardless of oil type ($P = 0.035$). Chroma (intensity of the color) also increased in meat from birds under FW ($P = 0.018$) and showed a triple interaction among HS, oil source, and FW, with the highest values observed in birds fed BSFO and subjected to FW under HS ($P = 0.042$). TBARS values did not show significant differences, although they increased numerically over time. In conclusion, HS during the finisher period impaired breast meat quality, reducing both WHC and lightness. The inclusion of BSFO combined with FW produced responses comparable to soybean oil under heat stress conditions, although specific changes in redness and chroma indicate a potential positive influence on color-related traits. This research was supported by grant PID2023-149145OB-I00 funded by MICIU/AEI/10.13039/501100011033 and ERDF/EU.

Beyond soybean meal: alternative grain legumes in diets for broiler chickens

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In the EU, recent efforts to reduce soybean meal use in poultry diets are driven by import reliance and environmental sustainability, while supporting the use of local protein sources. Among these alternatives, grain legumes have gained attention, yet their effects on chicken performance and meat traits remain underexplored. Therefore, this study evaluated the effects of partially replacing soybean meal with legumes (faba bean, Vicia faba var. minor; or white lupin, Lupinus albus) and sunflower meal on the performance, slaughter results, and rheological traits of broiler chickens. From hatch to 36 d of age, a total of 624 male chickens housed in 24 pens (26 birds/pen; 6 pens/treatment) were fed either a commercial soybean-based diet (C) or the same diet including faba bean (F; 90% C + 10% faba bean), lupin (L; 90% C + 10% lupin) or dehulled sunflower meal (S; 90% C + 10% sunflower meal). Slaughter traits, breast myopathy occurrence, and pH, colour, thawing and cooking losses, and shear force of pectoralis major were assessed on 144 birds (6 birds/pen; 36 birds/treatment). Daily weight gain was lower in chickens fed the experimental diets compared with the commercial diet ($p<0.001$), resulting in lower final live weight in chickens fed diet F (−10%) and diets L and S (−14% and −16%, respectively) ($p<0.001$), whereas feed conversion ratio was higher in chickens fed the experimental diets (+14% on average) compared to diet C ($p<0.001$). Carcass yield was unaffected (71.9%, on average), whereas breast yield was highest with diet F (40.4% cold carcass weight), intermediate with diets C and L (39.7 and 38.6%), and lowest with diet S (37.4%) ($p<0.001$). As for breast myopathies, white striping occurrence increased from 2.8% with diet S to 13.9% and 16.7% with diets L and F, reaching the highest rate with diet C (25.0%; $p<0.05$). Wooden breast occurrence increased from 0% with diets L and S to 5.6% with diet F and 13.9% with diet C ($p<0.01$); spaghetti meat occurrence was not affected by diet (1.4%; $p=0.42$). Regarding meat quality, p. major pH was lower (−3%) and lightness (L*) higher (+6%) with diet S compared with diet C ($p<0.001$), whereas yellowness (b*) was lowest with diet F and highest with diet S ($p<0.001$). No significant effects were observed for redness (a*), thawing losses (13%), cooking losses (25%), or shear force (1.95 kg/g). In conclusion, partial replacement of soybean meal with grain legumes or sunflower meal resulted in reduced growth performance, with the faba bean diet being closer to the commercial diet than the other experimental diets. The lower growth rate associated with partial soybean meal replacement likely contributed to reduced white striping and wooden breast occurrence, while only minor changes were observed in meat quality traits. This study was funded by the Project “Strategie di adattamento ai cambiamenti climatici: introduzione e valorizzazione nell’areale veneto di nuove leguminose per l’alimentazione umana e animale”, offered by the University of Padova, DAFNAE Department (Reference: 2024DAFNAE1SIDPROGETTI-00026).

Programmable automated dispenser for high-frequency delivery of dried black soldier fly larvae as behavioural enrichment in broilers

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Fast-growing broilers exhibit decreased locomotor activity, which is associated with impaired welfare and leg health. Provision of black soldier fly larvae (BSFL) has been shown to stimulate foraging behaviour and increase activity, but these effects are short-lived when larvae are offered infrequently. Sustained behavioural stimulation requires repeated delivery of small quantities throughout the day, which is difficult to manage manually. Accurate timing and portion control are therefore essential for using BSFL as an edible enrichment in a way that supports both locomotion and welfare. The objective of this study was to develop and technically validate a programmable dispenser capable of delivering dried BSFL frequently and on a set schedule in broiler barns. It was hypothesised that a network-synchronised dispenser would provide accurate and reliable BSFL delivery during the trial. The dispenser had a rotating disc with 41 compartments, each holding a prefilled amount of dried BSFL for 40 feeding portions. A stepper motor ensured accurate movement and portion release. Feeding events were controlled by a microcontroller-based unit, with programmable parameters adjustable via a configuration file. Synchronisation via Network Time Protocol (NTP) ensured accurate time control, independent of power interruptions. In a pilot experiment, broilers received BSFL approximately 10% of their daily feed intake for a full fattening period of 42 days. The larvae were dispensed at 20-minute intervals from 07:30 (GMT+2) during a 13-hour light period. The system operated semi-autonomously in an experimental rearing pen. The system demonstrated robust mechanical performance, delivering consistent portions with accurate temporal control. The system executed 1,680 feeding events without mechanical failure or considerable time deviation. Broilers consistently approached the dispensing area following release events, indicating acceptance of the BSFL as feed and behavioural enrichment. Activity levels remained elevated throughout the five-week observation period of the six-week trial, with no observable habituation to the repeated feedings. Further analysis is underway to measure the full effects on behaviour and performance. Overall, the developed system represents a practical and scalable technical solution for integrating BSFL into broiler feeding and welfare strategies while enabling long-term enrichment

Italian consumers' concerns about the use of insect-derived products in ruminant feed: evidence from CUB models

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The transition towards more sustainable livestock systems requires alternative protein and lipid sources that can reduce environmental pressure and competition between food and feed. Due to their nutritional value and resource efficiency, insects are increasingly recognised as promising feed ingredients. However, despite regulatory developments within the European Union, the use of insect-derived products in ruminant feeding remains limited and could raise specific consumer concerns that might hinder market development. Understanding the structure and determinants of these concerns is relevant for the livestock sector. This study tests two hypotheses: (H1) that Italian consumers express significant concerns about the use of insect-derived products in ruminant diets, but with varying levels of uncertainty in their responses; and (H2) that the intensity of concern and associated uncertainty vary according to socio-demographic characteristics. Data were collected through a survey of 1,040 Italian consumers. Respondents evaluated eight items relating to their level of concern using a 7-point Likert scale (1 = "not at all concerned"; 7 = "extremely concerned"). The items covered inadequate communication to consumers, traceability, hygiene and safety standards, food price, labelling transparency, availability of insects, insect rearing practices and insect processing methods. CUB (Combination of a discrete Uniform and a shifted Binomial distribution) models were estimated via maximum likelihood to distinguish the "feeling" component (systematic inclination towards higher concern levels) from the "uncertainty" component (random response behaviour). The models were first fitted to the overall sample and were then extended to include age and degree of urbanisation (continuous covariates), the statistical significance of which was tested within both components. The results confirmed H1: very high levels of concern emerged for all items (1 – ^ > 0.80), except for sufficient insect availability (1 – ^ = 0.04). Uncertainty parameters were consistently high (1 – ^ > 0.70), indicating widespread hesitation or limited knowledge about this innovation. Regarding H2, age was found to significantly affect all items: concern increased with age, while uncertainty decreased. This suggests that older consumers have more defined attitudes. Urbanisation had different effects: higher levels of urbanisation were associated with greater concern about communication, traceability, and labelling transparency, whereas lower levels of urbanisation were linked to higher concern about insect availability and processing methods. A significant effect of urbanisation on uncertainty emerged only for insect processing methods, with uncertainty increasing in more urban contexts. Overall, the findings consistently show high levels of concern regarding the use of insect-derived products in ruminant feeding, and confirm that both the intensity of concern and the uncertainty of responses are systematically shaped by socio-demographic factors. The high uncertainty parameters are consistent with the risk perception and knowledge gap dynamics that are typically associated with novel feed innovations. These results highlight the importance of transparent communication and evidence-based information strategies in supporting the acceptance of sustainable feed solutions in the ruminant sector. Research supported by Hi-Ga project; Green Era Hub on Agri Food and Biotechnology.

Protein-rich, locally sourced feed ingredients efficiently utilized by young chicks

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As part of the CASDAR PRESAJE project, which aims to improve our understanding of the mechanisms related to chick (0-10 old days) nutrition and health, the hypothesis that early-life digestive health may be improved by reducing dietary protein intake during the first day's post-hatch was investigated. In this context, the use of locally sourced feedstuffs that are lower in protein and richer in fibre as alternatives to soybean meal could represent a viable solution. Such an adapted starter diet would facilitate the transition to a more diversified diet during the growth phase. However, scientific references regarding the digestive utilization of raw materials by very young animals remain infrequent. For this reason, ARVALIS conducted several trials to evaluate the digestibility of various high-protein raw materials (HPRM) and thereby determine their nutritional value (energy, protein, starch, amino acids). Ten raw materials were evaluated in ROSS 308 and the nutritional values were obtained by difference calculation using the values of a complementary diet. This one was given alone for several birds and mixed with the tested raw materials for the other birds. The digestibility balance was conducted over three days, from day 5 to day 8. At this age, excretion levels are very low; therefore, animals were housed in pairs. Given the low amount of excreta of the young chicks, their chemical composition was predicted using Near-Infrared Spectroscopy (NIRS), based on calibrations developed by ARVALIS. Amino acid analyses were performed at the faecal level, whereas amino acid digestibility is usually assessed either in caecectomized roosters or through ileal sampling in chickens. This methodological choice was deliberate, as we consider the hypothesis of a negligible effect of caecal modifications at this age to be plausible. In comparison with defatted soybean meal (SBMdef), several expeller meals – two rapeseed expeller meals (RSMexp), two sunflower expeller meal (SFMexp), and two soybean expeller meals (SBMexp) – were assessed, along with dehulled faba beans (FBdeh) and peas (Pdeh) and toasted soybean seeds (SBStos). Across all tested HPRM, crude protein content ranged from 23 (Pdeh) to 49% DM (SMexp), and NDF levels ranged from 7.8 (FBdeh) to 25% DM (RSMexp and SFMexp). Regarding apparent crude protein digestibility, the best-utilized HPRM were FBdeh (92.3%), SBStos (88.3%), and both SBMexp samples (88.1% on average). Semi-dehulled RSMexp showed the lowest nitrogen digestibility (75.3%). Energy utilization of these HPRM ranged from approximately 2000 kcal/kg DM for SFMexp and semi-dehulled RSMexp to 3748 kcal/kg DM for toasted soybean seeds. For the use of a SBMdef, the impact of a higher degree of dehulling (90% versus 60%) resulted in an improvement in AMEn values of 209 kcal/kg DM, along with increases of 9.1 points in nitrogen apparent digestibility and 2.0 points in energy apparent digestibility. Regarding amino acid digestibility, and lysine in particular, the highest values were observed for FBdeh (94.5%) and for one RSMexp (91.6%), whereas the lowest value was recorded for semi-dehulled RSMexp (78%). These results clearly demonstrate the large diversity of HPRM, many of which are efficiently utilized by very young birds and could therefore be incorporated into starter diet formulations for chicks, with the objective of promoting adaptation to diverse and locally sourced feedstuffs during the starter period. These reference values were used to formulate diets in performance trials.

Impact of BSF Larvae Inclusion on Pig Growth

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The growth of the world's population results in an increased demand for animal products, placing increasing pressure on conventional feed ingredients such as soybean meal and fish meal. The use of insect meal is an opportunity to reduce the pressure on raw materials for animal feed and "Food vs Feed" competition, leveraging a new sector with great potential. This study is being conducted under the Mobilizing Agenda InsectERA (AAC nº02/C05-i01/2022). The study aims to evaluate the added value of defatted BSF larvae meal in growing pigs feed, considering zootechnical performance. The trial was conducted in the UTAD facilities, and was divided in two phases: In the 1st phase 18 litters were selected (6 replicates/treatment; 6 animals/replicate), from which 72 male piglets were selected for the 2nd phase. Piglets' average weight was 3.2 kg at 8 days of age.The 2nd phase of the trial was divided in two sub-phases:from 28 to 42 days (pre-starter diet). Each treatment had 8 replicates for consumption (measurements by pen; 3 animals/pen) and 24 replicates for growth (measurements by animal; 24 animals). Afterwards 8 animals per treatment were slaughtered. This data was presented at EAAP Annual Meeting in 2025.from 42 to 63 days (starter diet). Each treatment had 8 replicates for consumption (pens) and 16 replicates for growth (animals). A total of 48 growing pigs with an average initial body weight of 12.104 kg were distributed to three feeding groups, Control (0% BSF larvae meal), T2 (2.5% BSF larvae meal) and T3 (5.0% BSF larvae meal). Diets (starter diet) were isonitrogenous and isoenergetic.The measurements were individual live weight (LW), individual average daily feed intake (ADFI) of feed, average daily gain (ADG), feed conversion ratio (FCR), morbidity and mortality during the period of the trial. There were no differences in LW, at the end of this phase of the trial, which averaged 25.008 kg. The ADFI, ADG and the FCR at this stage did not show significant differences between the experimental treatments, either over the weeks or over the entire period, averaging 0.946 kg/day, 0.6149 kg and 1.549, respectively. As conclusion we realised that the inclusion of 2.5% and 5% in the starter diet had no relevant positive or negative effects on the productive response of piglets under the conditions of our trial.

Effects of Replacing Plasma Meal with Defatted Black Soldier Fly (Hermetia illucens) Larva Meal on Post-Weaning Piglet Performance, Blood Parameters, and Histopathology

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Black soldier fly (Hermetia illucens) larvae have been used in swine nutrition due to their high content of protein, vitamins, minerals, essential amino acids, polyunsaturated fatty acids, and bioactive compounds such as chitin and antimicrobial peptides. Animal production seeks sustainable strategies to improve efficiency, and insects can play an important role in this context. The weaning period is critical for piglets and is often associated with post-weaning diarrhea caused by Escherichia coli. H. illucens larvae have been studied as a feed ingredient due to their nutritional value and antimicrobial potential. This study aimed to evaluate the effects of replacing spray-dried plasma (SDP), a feed ingredient with high protein content, digestibility, and biological value widely used in piglet diets, with defatted BSF meal (DBSFM) in post-weaning piglet diets. The trial included two experimental treatments: 1) Control, with pre-starter and starter diets containing 2.5 and 3.0% SDP, respectively; and 2) DBSFM, with pre-starter and starter diets containing 3.1 and 8.35% DBSFM, respectively. Diets were formulated to be iso-nitrogenous and iso-energetic for each feeding phase. A total of 48 piglets ((LW × LR) × Pi) were randomly allocated to the two treatments and housed in pairs in 24 nursery pens for five weeks. Piglets were weighed weekly, and feed intake was recorded to determine average daily gain (ADG), average daily feed intake (ADFI), and feed conversion ratio (FCR). No significant differences were observed in initial body weight (6.500 vs. 6.302; P=0.4743), final body weight (18.168 vs. 19.028; P=0.3827), ADG (0.331 vs. 0.361; P=0.1895), or FCR (1.803 vs. 1.747; P=0.6446). However, pigs fed the BSF diet showed higher ADFI (0.576 vs. 0.668; P=0.0404). No significant differences were observed between treatments for most hematological and biochemical parameters (P>0.05). However, pigs fed DBSFM showed lower eosinophils (0.375 vs. 0.917; P=0.006), basophils (0.883 vs. 1.442; P=0.029), and absolute eosinophils (0.064 vs. 0.177; P=0.002) compared with pigs fed plasma. In the erythrogram, hemoglobina (10.225 vs. 11.242; P=0.006), hematocrit (34.083 vs. 37.358; P=0.011), and MCH (15.825 vs. 16.492; P=0.044) were lower in the BSF group. In serum biochemistry, only magnesium (2.0 vs. 2.2; P=0.015) and phosphorus (9.2 vs. 10.2; P=0.014) differed between treatments. However, all values remained within the physiological reference ranges. The inclusion of BSF larva meal in place of plasma meal in piglet diets did not cause significant histopathological changes in the heart, lungs, kidneys, liver, or spleen (p > 0.05), suggesting the safety of the ingredient. It is concluded that the inclusion of defatted BSF meal in replacement of plasma meal in post-weaning piglet diets can be carried out without any negative effects. Future trials are suggested to increase sample size and to evaluate other inclusion levels. Keywords: BSF meal, plasma meal, performance, blood parameters, histopathology, piglets

Recovering missing calving events to improve lactation number accuracy in commercial dairy cow datasets

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Accurate information on lactation number (parity) is essential in dairy science as both milk yield and lactation curve shape depend strongly on parity. With the increasing availability of data from automated farm systems, large datasets can be used for research, but completeness and recording errors remain a challenge. Farms, even when using the same herd management system, may to various degrees record data about key events such as inseminations, pregnancy checks, calvings, dry off dates and culling decisions. Differences between expected and actual user behaviour may also lead to issues such as a new lactation, as recorded by the herd management system, beginning several days into the actual lactation period. This makes it difficult to accurately filter out dry off periods or dairy cows with high milk yields towards the end of their lactation. The objective of this study was to improve the accuracy of lactation number estimation by identifying and correcting common sources of error in calving registrations and by integrating multiple data sources. Data were obtained from 11 commercial dairy farms participating in the Gigacow infrastructure. The dataset included daily milk yield records, calving registrations and calf birthdates from the DeLaval DelPro herd management system, as well as calving information from the Swedish Official Milk Recording Scheme (SOMRS). Data were retrieved on 6 March 2026. Additional calving events were inferred using calf birthdates linked to their dam through individual identification numbers. The combined dataset was screened for erroneous registrations using biological plausibility checks. Calving intervals shorter than 42 days were considered erroneous, while intervals between 42 and 260 days were retained but flagged as likely abortions. Calving data were merged with milk yield records, and only cows with at least one positive milk yield observation were retained. To ensure temporal consistency, calving events were restricted to periods when milk yield recordings from each respective farm were available in Gigacow. Cow birthdates were used to calculate age at calving and age at first milking. Records with biologically implausible ages were inspected and removed when errors were detected. Data processing and validation were conducted in R using tidy data workflows. Visual inspection of timelines combining birthdates, calving events and milk yield records was used for visual validation of reconstructed lactation histories. Data from the herd management system with and without recovery of calving dates was compared with calving data from SOMRS. Preliminary results show that the initial DelPro dataset contained 75% (2443/3257) of the calving records in the SOMRS dataset. Cleaning of the DelPro dataset where improperly recorded national animal ID numbers were restored recovered an additional 340 calving events and resulted in 85% coverage. Finally, the integrated dataset including inferred calvings contained 87% (2836/3257) of the records in SOMRS. Further improvements are expected by incorporating additional checks based on daily milk yield patterns and age at calving. The proposed approach demonstrates how integrating multiple farm data streams can substantially improve data quality and enable large-scale dairy production research as data becomes available through new data standards and exchange services such as the ICAR Animal Data Exchange standard and the International Dairy Data Exchange Network (iDDEN).

Toward a digital model of dairy cow time budget and related energy expenditure

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Advances in on-animal and in-barn sensing technologies enable high-resolution behavioural monitoring. However, the utility of these data depends not only on accurate behavioural classification but also on the availability of predictive behavioural models capable of describing the expected temporal organization of activities under normal conditions. Such models can serve as benchmarks for detecting behavioural deviations. To date, very few mathematical models of dairy cattle behaviour have been proposed, and most have been designed with highly specific objectives, typically aiming to predict a single behavioural variable. There is therefore a need for integrative modelling frameworks capable of capturing the full structure of an animal's activity over time.This study proposes a mechanistic and dynamic model that predicts individual dairy cattle time budgets across physiological states, management contexts, and environmental conditions. The model assumes that animals allocate behaviour by minimizing deviations from behavioural needs, defined as daily requirements for feeding, drinking, milking, resting, sleeping, ruminating, social interaction, and environmental engagement. These needs are expressed in terms of target behaviour durations, computed on the basis of daily individual performance variables (milk yield, intake, body weight, etc.) predicted by a lifetime model of dairy cows (InSiliCow), combined with management cues (diet composition, outdoor access, etc.). Stochasticity and adjustable individual parameters (e.g., personality traits) generate inter-individual variability in targeted needs and sensitivity to constraints. Transitions between behavioural states arise from the interaction between internal motivation (unmet needs) and external opportunity (resource availability), while environmental factors (indoor vs. outdoor; parlour vs. robot), temporal cues (feeding schedules, milking, circadian phases), and social constraints (crowding, hierarchy) modulate the decision-making process. The model produces coherent time budgets and additionally estimates individual energy expenditure associated with physical activity.Overall, this framework offers a biologically grounded and flexible tool for exploring how intrinsic animal characteristics, environmental conditions, and management practices shape behavioural dynamics. It also provides a basis for detecting behavioural deviations, competition effects, and welfare-relevant disturbances over time.

Digital Transformation in Animal Assessment: A Mobile Application for Real-Time Data Collection and On-Site Analysis

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Manual data collection using paper forms remains a widespread practice in animal assessment, particularly in field studies, on-farm welfare evaluations, breeding programs, and long-term ecological or behavioral research. However, paper-based workflows require post-hoc transcription into digital systems, which is time-consuming, labor-intensive, and prone to entry errors. This delays analysis and can compromise data quality and reproducibility. Additionally, paper-based documentation may pose biosecurity risks, as forms handled on farms are brought into office environments where pathogens can spread to surfaces, materials, or personnel and subsequently be carried to other farms. To address these systemic challenges, we developed an offline-first mobile application designed for all major farm animal species (cattle, pigs, sheep, goats, poultry), suitable for research, breeding, and on-farm welfare assessments. The application enables fully digital data collection directly in the field. All data is stored locally on the device and is not automatically transmitted, ensuring data security and user control. A key innovation is integrated real-time data analysis. Prior to assessment, the application calculates required sample size, while optional trend monitoring and automated alerts flag outliers or critical values during data collection. Animals can be selected either randomly or automated (e.g., NFC or QR codes). Assessments may be conducted at individual or group level, depending on the study design. Following data entry, results are immediately visualized through interactive graphs, summary statistics and deviation indicators, enabling real-time quality control and detection of anomalies. The system features modular architecture, allowing new assessment criteria, scoring schemes, or image-based guidance to be integrated easily. This supports adaptation to diverse research objectives, genetic evaluations, and welfare assessments. By replacing paper-based workflows, the application reduces transcription effort and minimizes entry-related errors, thereby improving data consistency and reproducibility. It was developed as a non-commercial app with domain experts to ensure practical applicability. The system supports standardized data collection, facilitates data sharing within research teams, and enables tracking of individual animals. Overall, it integrates field assessment and data analysis into a coherent digital workflow for animal science research and on-farm evaluation.

Human classification of pig vocalizations shows limited context-independent emotional structure

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Pig vocalizations are increasingly used as indicators of affective states in research on emotional capacities. Such interpretations typically assume that animals exposed to similar experimental contexts experience comparable affective states and that vocalizations can be assigned to discrete emotional categories—presupposing biologically fixed emotional signatures inferable from acoustic structure alone. However, advances in affective neuroscience emphasize degeneracy, whereby similar behavioral outputs may arise from different internal processes, suggesting emotional categories may reflect observer-dependent interpretations rather than direct access to internal states. Previous studies have relied largely on forced- or multiple-choice paradigms, which may inflate classification accuracy. Few studies have examined how humans spontaneously group pig vocalizations without contextual cues. This study examined human classification of pig vocalizations using a free-sorting paradigm. Vocalizations were obtained from the Soundwel database and filtered using objective acoustic quality criteria (SNR ≥ 6 dB, duration ≥ 0.10 s, loudness ≥ −30 LUFS), yielding 3,144 calls from 18 experimentally defined contexts. In February 2026, 216 participants freely grouped 40 sounds (two per context plus controls) into self-defined categories online. Similarity matrices based on co-classification patterns were constructed at the context level, hierarchical clustering applied using hclust in R, and semantic analysis performed via WordNet through Python's NLTK interface within R. Within-context consistency was moderate (mean = 48.0%, SD = 16.9%) and varied substantially across contexts. Highest consistency was observed for negative conditioning (70.4%), positive conditioning (69.0%), and castration (61.1%); lowest for crushing (27.3%), novel object test (30.6%), and short reunion (30.6%). Hierarchical clustering revealed three broad clusters corresponding to handling-related (e.g., castration, restraint), social (e.g., nursing, huddling), and experimental or environmental contexts (e.g., enrichment, barren), with within-cluster similarity of 0.52–0.89 and between-cluster similarity of 0.10–0.71. The most distinct cluster contained vocalizations from castration, restraint, fighting, and crushing in piglets. Semantic analysis showed these contexts received the highest proportion of "feeling"-related labels (27–35%), predominantly negative in valence. Sounds from positive and lower-intensity negative contexts showed considerably more overlap, with mixed positive and negative semantic labels. Clustering patterns were highly similar across expertise levels ($r = 0.92–0.94$). Higher-expertise participants replayed sounds fewer times before classifying (−1.3 replays, $p < 0.0001$), but expertise did not improve classification consistency. Overall, humans showed only moderate ability to recover context-related structure without contextual information. Substantial overlap across contexts and limited influence of expertise suggest spontaneous perception relies on coarse acoustic heuristics rather than stable, context-specific vocal signatures, supporting a cautious interpretation of pig vocalizations as direct indicators of discrete emotional states.

Low cost IoT system for monitoring environmental conditions and behavior in pig production

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Livestock farming production have been using modern technologies and techniques in order to impact the sustainability of the animals production. Conventional livestock production systems, as well some research in different livestock areas have been using equipment and tools to monitoring the environment and Pig behavior, that are usually expensive, reducing the opportunities to be acquire either for small and medium producers and research applications in livestock production. Based on this context, a low-cost open-source IoT tool for monitoring pig behavior and environmental conditions in facilities production was developed. The system was validated and tested in a maternity pig farm, where the developed sensor network has provided reliable information as temperature, humidity, light intensity, temperature of the sows and piglets, black globe temperature, sound, CH4 concentration and records normal or abnormal animal behavior, and it could be used to support decision-making and technological processes in livestock production, as well in various research projects. Further adjustments specific variables will be implemented the second phase of this research.

Added value of multi-sensor behavioural monitoring in infectious disease trials: a case study with pigs challenged with African Swine Fever Virus

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Animal experiments, such as vaccine efficacy trials, can benefit from sensor-based behavioural monitoring. Compared to traditional (bi-)daily clinical observations, sensors provide 24/7 behavioural data that may allow for earlier detection of anomalies and for a more detailed assessment of differences between animals. Here, we explored the added value of sensor-based monitoring of activity and feeding behaviour in a vaccine efficacy trial with three groups of pigs challenged with African Swine Fever Virus. Four weeks before the challenge, group 1 (n = 6) had received a candidate vaccine orally, group 2 (n = 6) had received the same vaccine intramuscularly, and group 3 (n = 4) was mock-vaccinated. From one week before the challenge onwards, we used our recently developed tool RAMSMART (https://git.wur.nl/nlas-asg/ramsmart) to monitor individual animal activity in all three groups. RAMSMART uses a lightweight accelerometer that continuously computes the Vectorial Dynamic Body Acceleration (VeDBA) through edge computing and broadcasts this VeDBA once per minute through Bluetooth low energy to a nearby receiving device. In group 3, we also applied a RFID system in the feeder (with 4-7 reads per second) to detect feeding visits. A feeding visit was defined as a series of detections lasting for at least 3 seconds and at most 5 minutes, with a maximum gap in between subsequent detections of 10 seconds. On day 3 after the challenge, VeDBA levels significantly dropped in orally vaccinated group 1 and the mock-vaccinated group 3, whereas the intramuscularly vaccinated group remained on its normal activity level. This was in line with the onset of fever in the groups, and RAMSMART detected these anomalies one day earlier than the rise in (bi-)daily clinical activity scores. On day 5, the mean VeDBA in groups 1 and 3 had reduced to <30% of what it used to be before challenge. Similarly, the RFID-based number of feeding events and feeding duration dropped in group 3 from day 3 onwards, whereas clinical appetite scores only increased on day 4 after the challenge. In the week before the challenge, each pig of group 3 visited the feeder approximately 75 times a day (with total duration ~60 minutes), whereas on day 4 after the challenge this reduced to less than 10 times a day (in total < 5 minutes). Overall, we demonstrate the added value of sensor-based monitoring in clinical (vaccine) trials and recommend wider implementation of such technologies to improve the quality of experimental results and refine animal trials.

Application of Multimodal IMU Metrics to the Behavioral Assessment of Pigs

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Multimodal data streams are becoming increasingly prevalent in animal behavior research, offering new opportunities to capture behavioral states and events with greater temporal resolution and contextual depth. Common data stream sources include video cameras, audio recorders, global positioning system (GPS) trackers, and wearable inertial Measurement Units (IMUs). The latter incorporates accelerometers, gyroscopes, and magnetometers to provide 9-axis high-frequency primary sensor outputs for interpretation, and, when synchronized with video and/or audio recordings, allows for fine-scale temporal alignment between observed behavior and underlying movement dynamics. Several interesting metrics can be derived directly from the individual IMU component outputs. The accelerometer yields a measure of activity from the total three-axis acceleration. The gyroscope offers angular velocity and acceleration, and the magnetometer reports orientation across three axes. However, the ability to define multimodal metrics via calculations across outputs from different sensors can provide novel insights into animal behavior. For example, combining the accelerometer and magnetometer outputs enables tracking an animal's path via dead reckoning. Calculating across the accelerometer and gyroscope data allows for the determination of an animal's true movement independent of posture via gravity-compensated linear acceleration. While there are a variety of other multimodal metrics that can arise from sensor fusion (Linear acceleration, Gravity vector estimate, Orientation estimates [Pitch / Roll / Yaw], etc.),a convenient platform for applying these novel metrics to animal behavior has not been available, particularly for easily calculating and visualizing them in an interpretable context. Here, we present a workflow that starts with parsed raw IMU wearable outputs and ends with the simultaneous, time-aligned visualization of behaving sows equipped with animal-borne IMUs, and the multimodal metrics calculated from those IMUs' outputs. A single interactive environment enables the calculation and visualization of user-defined metrics from the IMU's accelerometer, gyroscope, and magnetometer outputs. A dedicated annotation software facilitates the labelling of multimodal data for behavioral states and events from concurrently coded IMU data and video recordings. These multimodal annotations are then visualized within a custom analytical interface developed in Power Query for relational structure and Power BI, allowing users to readily explore synchronized behavioral labels alongside calculated IMU metrics. By linking multimodal IMU orientation and amplitude metrics with annotated behavioral patterns, this approach provides deeper insight into behavioral transitions, a digital ethogram with states and events, and temporal structure that are often lost in traditional ethograms. Such multimodal visualization and fusion frameworks support more robust behavioral assessment and facilitate the development of biologically grounded machine-learning models.

Development of an Automated Posture Prediction System for Sows Using Artificial Intelligence

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Monitoring animal behavior is needed for detecting major physiological events in livestock farming and for assessing welfare. The objective was to develop an automated posture prediction device for loosed-housed sows using a Convolutional Neural Network, the YOLO object detection. The system, consisting of a minicomputer included in a waterproof central unit and connected to one to three cameras positioned above and behind the sow's pen to enable individual observation, was developed in our workshop and then tested under farm condition in INRAE experimental farm. The artificial intelligence model was trained on a database of 10,483 images including two different farms and conditions whether animals were housed on slatted floor or on straw and dozens of Large White sows, taken from any moment of the day, to recognise eight distinct sow postures, as well as the location of her head and the location of each piglet. Dedicated softwares were designed for system configuration, real-time data processing, and result visualisation. It can be controlled without internet using a digital device that can connect to a wireless connection. The processing and visualisation softwares are also available for use at the office with additional features. Initial results from on-farm testing show a significant correlation between postural activity and the approach of farrowing. Notably, a marked increase in the frequency of posture changes and the increased use of specific postural sequences were observed in the hours preceding the event. These behavioral variations, automatically quantified by the system for each sow (each camera), represent promising early indicators for predicting farrowing and identifying potential health issues. This computer vision system, encompassing both hardware and software components, demonstrates the potential of embedded artificial intelligence techniques for developing decision-support tools in precision livestock farming. Its flexible architecture also allows for future applications in other species, simply by retraining the model on the target species' behavioral data. It paves the way for non-invasive, continuous monitoring, ultimately improving both animal health and welfare, and farm management efficiency.

Automated computer vision analysis of pig appeasing pheromone effects on social behaviour and welfare in group-housed sows

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In the EU, the reproductive cycle of sows commonly includes prolonged periods of individual housing during the farrowing and insemination phase, followed by the mandatory group housing during the gestation phase. The regrouping of previously familiar and partly unfamiliar sows at the start of gestation triggers agonistic interactions for (re-)establishing a social hierarchy. This period of social unrest is associated with stress and a high incidence of lesions and lameness, posing welfare and productivity challenges. The aim of this study was to investigate whether social unrest and its negative consequences could be alleviated by the application of a Pig Appeasing Pheromone (PAP) during the first week of the gestation period. In a double-blind experiment, 8 groups of sows (16–20 per group) were randomly allocated to a PAP (9 diffuser blocks containing 2% SecurePig® distributed across the pen from 3d before until 7d after mixing) or placebo treatment (identical placement of diffuser blocks lacking the active PAP component) at the start of the gestation period. Skin lesions obtained after mixing were scored on d2, d7, d9 and d17. Four cameras per pen were used to video-record the uniquely marked sows continuously from 8-20h for 17d after mixing. An in-housed developed and validated automated computer-vision-based system was used to enable high-resolution, continuous tracking and analysis of individual and dyadic behaviour. Zero-shot-learning-based segmentation, identification, and tracking enabled quantification of movement and agonistic interactions. Our AniDomNet tool was fitted separately to sequential fleeing and displacement interactions to infer social dominance matrices and directed acyclic graphs. Effects of treatment were tested using linear mixed models. PAP was associated with reduced overall movement ($p = 0.002$), fighting ($p = 0.011$), fleeing ($p < 0.001$), displacements ($p < 0.001$), and skin lesion scores ($p = 0.046$). The largest reduction occurred between d2 and d7 post-grouping, following the initial peak in agonistic behaviour immediately after mixing. These effects were sustained following PAP diffuser removal at d7 without signs of a rebound effect. The AniDomNet hierarchies based on fleeing vs displacements showed 65% agreement, and indicated a broadly linear dominance structure with some unsettled dyads. The effects of PAP on hierarchy structure seemed marginal: PAP groups showed slightly less linear hierarchies (using Landau h) and less settlement at lower ranks. High-ranked animals had more strongly preferred resting places, were on average further away from the feeding station, moved less (especially in control groups), were closer to each other during resting, and occupied areas less equally as compared to lower-ranked sows. This study demonstrates that PAP administration can reduce social stress during early gestation grouping, and that integrating zero-shot learning with continuous tracking provides a scalable, observer-independent approach for advancing ethological research in group-housed farm animals.

Camera-based computer vision monitoring of pig behaviour under heat stress

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Animals' exposure to high temperatures increases the risk of heat stress. Precision livestock farming (PLF) tools, such as camera-based computer vision, enable continuous, non-invasive monitoring of animal behaviour and can help detect heat stress at an early stage. This study aimed to assess behavioural responses of fattening pigs to heat stress using video-based analysis. The study was conducted at the CITA-IVA facilities (Segorbe, Spain) from 19 June to 18 July under commercial conditions. Here eight cameras were installed in top view in eight pens, each with a different floor type: four with fully slatted floors and four with partially solid floors with stirring fans. Each pen housed eight pigs and was continuously monitored. Temperature and humidity were continuously recorded using Dräger sensors, and the temperature–humidity index (THI) was calculated to quantify heat load. A total of 500 labelled images from our recordings were used to train the model for our experimental setup. Videos were analysed at 1 frame per second using the M3PIG pipeline (https://gitlab.kuleuven.be/m3-biores/public/m3pig). Rotated bounding boxes, ten keypoint positions (from snout to tail base), and posture (lying on the belly, right and left sides, sitting, standing) were also extracted. Space use (solid vs slatted floor), feeding and drinking behaviour were derived from annotated regions of interest, allowing the identification of animal position relative to floor type, feeders, and drinkers. Preliminary results showed that pigs spent most of their time lying (83.1%), with standing and sitting accounting for 15.2% and 1.7%, respectively, reflecting pigs' reduced activity in hot conditions as a thermoregulatory response. Lying time increased ($r = 0.2$), standing decreased ($r = -0.2$), and feeding time decreased ($r = -0.3$) with increasing THI. These behavioural changes may contribute to differences in emission dynamics reported in partially solid flooring systems. Increasing THI was also associated with greater use of the solid floor ($r = 0.32$) and less use of the slatted floor (-0.32), indicating a shift toward cooler areas under heat stress. These behavioural changes demonstrate that posture, floor use and feeding are sensitive indicators for automated detection of heat stress in pigs, supporting their integration into PLF tools for early management decision-making.

Zero-shot computer vision-based analysis of social behaviour among group-housed sows

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In group-housed sows, dominance hierarchies are established through frequent, often brief agonistic interactions. Quantifying reliable dominance dynamics requires high-resolution, individual-level monitoring of behaviour. Traditional behaviour scoring is often limited by short observation periods and constrained by observer subjectivity. Existing automated approaches often depend on human annotations for supervised models and require pen-specific tuning, limiting transferability across environments and experiments. Here, we present the development and validation of a scalable computer vision pipeline for long-term automated monitoring of group-housed sows using zero-shot learning, enabling quantification from movement patterns to dominance hierarchies with reduced reliance on pixel-level annotations. Four ceiling-mounted cameras per pen captured continuous video of 16–20 uniquely marked sows per group over a 17-day observation period. These video recordings were analysed at the frame level using a zero-shot segmentation pipeline that combined DINO-based self-supervised features, a U-Net refinement step, and SAMv2 mask proposals. Individual (re-)identification was performed using a supervised MaxViT network trained on unique marking patterns, where confidence-weighted predictions were aggregated within tracks to reduce identity switches. Multi-camera detections were aligned on a common timeline and filtered using mask geometry to mitigate peripheral lens distortion. Tracklets were formed using spatiotemporal continuity and automatically linked across pen sections using pen geometry. Occlusions and short recording gaps were handled using conservative track termination and hourly stitching, without interpolating missing positions. A grid-based post-processing step was applied to further reduce jitter and improve the stability of spatial position estimates for individual sows. Three agonistic interaction types (fighting, fleeing, and displacement) were modelled using a rule-based classifier based on trajectories and dyadic configurations. These interactions were used to infer group-level dominance dynamics with AniDomNet, a probabilistic dominance inference model that estimates a dominance matrix from sequential dyadic interactions and constructs a directed acyclic graph to derive rankings. Dominance hierarchies were inferred separately for fleeing and displacement behaviours. The track precision when validated against manual annotations showed high robustness, reaching 96.6%. The (re-)identification network achieved a validation accuracy of 98.6%. Detection precision for agonistic interactions was 0.82 for fighting, 0.73 for fleeing, and 0.77 for displacement. Overall, integrating zero-shot learning with multi-camera tracking enabled scalable, observer-independent behavioural quantification in group-housed farm animals, with improved transferability to new pens.

Moo4feed R package: Decoding individual animal narratives through precision livestock monitoring data

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Precision Livestock Farming (PLF) technologies enable continuous, automated monitoring of individual animals, yet most data analysis still relies on group-level comparisons that omit meaningful individual differences. There is a growing acceptance that animals housed in identical environments exhibit different behaviors even when healthy, thus for accurate and timely detection of health or welfare challenges it is important to track within animal changes, rather than applying herd-level thresholds and a 'one size fits all' treatment. The aim of this study was to extract existing PLF data streams to identify novel behavioral variables and characterize individual-level behavioral consistency in dairy cows. Data were collected from 159 lactating Holstein dairy cows at the UBC Dairy Education and Research Centre over 10 months. We applied strict data cleaning and filtering to exclude periods of illness, lameness (based on two consecutive non-lame weekly scores), estrus, technical outage, and regrouping events, which resulted in a total of 73 cows across 47 days within 3 stable periods. We identified 21 behavioral variables derived from electronic feed bin and water trough (Insentec, NL) data. Beyond conventional intake metrics (e.g., feed and water intake, duration, meal frequency), we extracted variables capturing exploratory behavior (e.g., non-nutritive visits), social dynamics (e.g., rate of initiating or receiving agonistic displacements per meal), and indicators of feed access quality (e.g., proportion of meal time spent actively eating versus waiting, proportion of fresh feed remaining upon arrival). Bayesian mixed-effects models were used to quantify repeatability (CVi; between-individual variation in average behavioral expression) and predictability (rIIV; residual intra-individual variance). Non-nutritive visits per meal (CVi = 0.676, rIIV = 2.330), proportion of actor displacements per meal (CVi = 0.638, rIIV = 2.065), total non-nutritive visits per day (CVi = 0.622, rIIV = 2.170), proportion of reactor displacements per meal (CVi = 0.469, rIIV = 2.024), median water visit duration (CVi = 0.412, rIIV = 2.637), and median visits per meal (CVi = 0.377, rIIV = 1.925) all exhibited high between-individual variation alongside low residual intra-individual variance, indicating these behaviors stay relatively consistent within individuals but differ between individuals. To support full reproducibility, we developed the moo4feed R package (www.skysheng.io/moo4feed/), a well-validated (96% test coverage) data science workflow with detailed tutorials for extracting these behavioral variables from raw PLF data, enabling future work to verify and build upon our methods. These findings demonstrate that in the case of automatic feeding and drinking systems there is tremendous potential to capture additional behavioral information beyond what is typically reported. Promoting individual-based behavioral profiling in dairy cattle, enabled through PLF technologies, is an important first step to developing new ways of improving early disease detection, affective state assessment, and personality characterization.

Influence of incomplete milking on the oxidative stress status in dairy cows during peak to mid lactation

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High milk production may represent a metabolic challenge in dairy cows. Previous studies demonstrated that automated short-term incomplete milking (IM) has the potential to alleviate metabolic load without impairing udder health or animal welfare indicators. The objective of this study was to investigate the effects of IM on oxidative stress status in multiparous Holstein cows during peak to mid lactation. As digital innovation for IM, a specialized software that precisely controlled milk withdrawal was implemented. A total of 46 multiparous Holstein cows housed under standardised conditions were included in a controlled experimental design. Twenty-three cows were assigned to the IM group, in which IM was implemented according to the individual milk yield using a software module that enabled automated early cluster removal. During the first phase of IM, milk withdrawal was gradually reduced by 9%/ day for 5.5 days to achieve an approximate 40% reduction. In the second phase of IM, milk withdrawal was maintained at the approximate 40% reduction in a constant manner for an additional 5.5 days. Two weeks before and after the IM period, IM cows were milked equally to control cows (CON group, n = 23), which underwent milk flow-based cluster removal at <0.3 kg/ min). Blood samples were collected weekly from the Vena Jugularis and plasma were analysed photometrically for indicators of oxidative stress, including derivates of reactive oxygen metabolites (dROM), advanced oxidation protein products (AOPP), thiobarbituric acid reactive substances (TBARS) and ferric reducing ability of plasma (FRAP). Additionally, the oxidative stress index (Osi = dROM / FRAP * 100) was calculated for each cow. Data were analysed using a linear mixed – model including treatment, time, treatment × time interaction, number of lactation and milk yield groups (> 40 kg vs. < 40 kg ECM / d) as fixed effects, and cow and days in milk at the beginning of the treatment as random effects. Alpha error correction for multiple comparisons was applied using the Bonferroni method Statistical significance was set at $P \leq 0.05$ and values are shown as mean ± SE. Oxidative stress indicators were comparable between groups and remained stable over time: (a) dROM: IM group 99.2 ± 3.6 µg H2O2 equivalent/mL, CON group 103.6 ± 3.6 µg H2O2 equivalent/mL; (b) AOPP: IM group 1.04 ± 0.09 µmol/g Protein, CON group 1.07 ± 0.02 µmol/g Protein; (c) TBARS: IM group 311 ± 5.3 nM, CON group 289.3 ± 3.6 nM; (d) FRAP: IM group 251.7 ± 2.4 µM Fe^{2+}/L, CON group 258.9 ± 2.6 µM Fe^{2+}/L; (e) Osi: IM group 40.01 ± 1.5, CON group 40.7 ± 1.3. No significant effects of IM were detected for any oxidative stress indicator ($P > 0.05$), neither between treatments, nor across weeks within a treatment group. In conclusion, automated short-term IM did not affect oxidative stress status in multiparous Holstein cows during peak to mid lactation, suggesting that the previously observed reduced metabolic load induced by IM does not translate into altered oxidative cell stress.

Automatic detection of cattle vocalizations based on spectrogram correlation, eigensounds, and deep learning embedding approaches

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The use of vocal indicators represents a unique and non-invasive means of assessing farmed animals' behaviour and welfare. The current study aimed to develop and evaluate the performance for a number of three detection approaches in order to automatically identify cattle vocalizations from audio recordings, as follows: i) spectrogram correlation using Praat and Parselmouth, ii) eigensounds analysis based on Principal Component Analysis, and iii) deep learning embeddings using the pre-trained YAMNet model. For this reason, we used curated audio-recordings from 20 Romanian Holstein lactating multiparous dairy cows, each monitored individually in a tied stanchion barn, following visual isolation of 1-hour post-morning milking. To assess and compare performance of the detection approaches, we evaluated the median precision and recall across all recordings. The spectrogram-correlation achieved a modest performance, with a precision of 0.53 and a recall of 0.28, reflecting most likely sensitivity to background environmental noises. The eigensounds approach had a moderate precision of 0.57 and a recall of 0.72, indicating thus a stronger sensitivity to factual vocalizations. In contrast, the YAMNet based embedding method offered the highest precision, of 0.88, all while maintaining a recall of 0.58. Current findings suggest that the three approaches can play distinct and synergistic roles in the development of future acoustic monitoring systems. This work targeted the automated detection and segmentation of individual vocalizations emitted by cattle, under farming conditions. Manual annotation of cattle vocalizations, although accurate when trained technicians are being used, requires intensive and time-consuming labour, while being difficult to keep consistent when hours long audio files are concerned. Therefore, throughout automatization of this step, two potential benefits arise, firstly it enables to work with significantly larger audio-sets, and secondly, makes real-time on-farm surveillance plausible, with no or little human-assistance needed during routine operations.

Gender-Related Differences in Growth Performance, Carcass Traits and Blood Parameters of Finishing Pigs

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Gender remains a key factor influencing growth performance, carcass traits and metabolic profile in pigs, particularly during the finishing phase. Within this framework, the present study aimed to assess sex-related differences in productive performance, backfat thickness and blood parameters in commercial pigs during the last four weeks of finishing. The study was conducted at UTAD Experimental Pig Farm facilities in Vila Real. Twenty-four Landrace × Large White pigs (12 males and 12 females) with initial body weight of 81.2 kg for females and 82.9 kg for males were allocated in individual pens and fed balanced diets under ad libitum conditions. Feed intake and live weight gain were monitored weekly. At the end of the trial, subcutaneous fat was evaluated at the rib level using a UTR device. Animals were sent to an official slaughterhouse, and at the time of slaughter, individual blood samples were collected and analysed to determine complete blood count and serum biochemistry. Data were analysed using ANOVA with gender as the main factor, and Tukey test was used for multiple comparisons of means. Statistical analyses were performed using JMP Pro 17. At the end of the finishing period, males exhibited significantly higher final body weight compared to females (110.0 vs. 103.7 kg; $p = 0.007$). Average daily gain followed the same trend, being greater in males (113.5 vs. 103.7 g/day; $p = 0.006$). No significant differences were observed in average daily feed intake ($p > 0.05$), while feed conversion ratio was markedly better in males (3.30 vs. 3.84; $p = 0.0001$), indicating superior growth efficiency. Backfat thickness tended to be higher in females (11.7 vs. 10.4 mm; $p = 0.096$). Serum biochemical analysis revealed no significant sex effects on GGT, ALT, AST, alkaline phosphatase or total bilirubin ($p > 0.05$). However, females presented higher total cholesterol (75.8 vs. 64.7 mg/dL; $p = 0.046$) and triglyceride concentrations (49.1 vs. 40.5 mg/dL; $p = 0.024$). Regarding haematological parameters, haematocrit percentage (46.05 vs. 42.53%; $p = 0.0061$) and haemoglobin concentration (14.23 vs. 13.03 g/dL; $p = 0.0145$) were significantly higher in females, while other parameters (erythrocytes, leukocytes, platelets, leukocyte formula, and mean corpuscular haemoglobin concentration) were not affected by sex. Overall, these findings confirm that biological sex significantly modulates growth efficiency and lipid-related metabolic traits in finishing pigs. Males demonstrated superior productive performance, whereas females exhibited a more pronounced lipid and erythroid profile. These differences should be considered when interpreting physiological responses in finishing pigs and in the optimisation of finishing management strategies. Keywords: Finishing pigs, sex differences, blood parameters Acknowledgments: This work was supported by the projects UID/00772/2025, DOI https://doi.org/10.54499/UID/00772/2025 funded by the Portuguese Foundation for Science and Technology (FCT).

Automated Monitoring of Rabbit Activity Using Computer Vision: A Comparison of Gaussian, SAM3, and YOLO Algorithms

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The management of commercial rabbit farming currently faces significant operational challenges, prominently the difficulty of detecting and analyzing rabbit activity in real-time. In this context the continuous monitoring of rabbit activity is time consuming, but key for detecting specific movement patterns that serve as early indicators of the animal's welfare and physiological state, such as identifying productive periods or detecting early signs of stress before they impact animal health. To address this, this study evaluates and implements multiple computer vision methodologies to quantify rabbit movement under standard husbandry conditions. Data acquisition was conducted at the experimental rabbit farm of the Universitat Politècnica de València (Eastern Spain). Video data was captured continuously over one month using top-view cameras. The experimental setup consisted of 8 individual cages (58×40 cm), housing one rabbit each, and two collective group-housing pen (176×176 cm) with 45 rabbits to monitor free interaction. Initially, a traditional, lightweight approach was tested using a Gaussian Mixture Modeling (GMM)-based background subtraction function (BackgroundSubstractorMOG2) via OpenCV. While effective for basic overall motion detection, more advanced segmentation was required in order to identify additional activities. Consequently, the heavy-computational Segment Anything Model 3 (SAM3) was implemented. Relying only on a prompt for rabbit identification, SAM3 provided highly reliable segmentation and tracking; however, its high computational cost made processing long videos difficult and restricted real-time, on-farm application. To overcome these processing demands, an efficient pipeline was developed. SAM3 was utilized strictly as an automatic annotation tool to precisely segment and label rabbits in their housing conditions with human verification. To enrich the model's predictive capacity, frames with high uniqueness were preselected for this auto-generated dataset, which was then used to train a computationally lighter object detection model (YOLO26). Preliminary results indicate that while the GMM approach effectively measures general movement, SAM3 offers the highest potential for segmentation at the cost of deployability. On the other hand, YOLO26 achieved reliable detection and faster inference speeds. It also demonstrated the capability to classify specific activities, such as visiting feeders or drinkers, and measure overall speed and movement in the collective pens. Ultimately, this methodology demonstrates that distilling high-load foundational models like SAM3 into efficient models like YOLO26 provides a viable, scalable solution for real-time activity measurement in precision livestock farming.

Can piglets self-regulate their intake when offered two contrasting diets differing in protein and energy?

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Precision feeding has emerged as a promising nutritional strategy in modern swine production, aiming to closely align nutrient supply with the individual requirements of animals across physiological stages. In piglets, whose nutritional needs are particularly dynamic during the post-weaning period, precision feeding helps limit the oversupply of costly nutrients such as amino acids and phosphorus, thereby reducing nitrogen excretion and the environmental footprint of pig production. Previous studies have shown that precision feeding systems based on real-time sensors and automated feeding technologies can maintain or even improve growth performance—including average daily gain (ADG), feed conversion ratio (FCR), while reducing feed costs by up to 10–15%. Beyond imposed dietary regimens, pigs exhibit active feed preferences linked to macronutrient composition, consistent with the concept of nutrient-specific appetites. When offered dietary choices, piglets are able to self-select macronutrient intakes that approximate a regulated intake target, prioritizing protein under limiting conditions or favouring energy-dense diets when energy demands are high. The present study investigated the dietary choices of group-housed piglets given simultaneous access to diets differing in net energy (NE) and crude protein (CP) content, with the objective of characterizing macronutrient selection patterns and assessing the nutritional and performance consequences of voluntary feed choice. To this end, 80 male and female piglets equipped with RFID tags were housed in four group pens and provided access to two automatic feeders capable of recording feed intake, number of visits to the feeder, and time spent at the feeder. One NE (kcal/kg) / CP (%) diet pair was evaluated under free-choice conditions in each pen, with the individual animal considered as the experimental unit. A control treatment consisted of the same diet offered in both feeders (2260/18.5), while three levels of nutritional contrast were tested under free-choice conditions: High (2400/13.5 and 2120/23.5), Moderate (2344/15.5 and 2176/21.5), and Low (2316/16.5 and 2204/20.5). If piglets consumed both diets in a 50:50 ratio, the resulting intake would be nutritionally equivalent to the control diet. Over the experimental period, piglets receiving the control diet consumed similar amounts from both feeders. In contrast, in pens offering contrasted dietary choices, animals showed a clear preference for energy-rich diets from the first week until the end of the experiment. After three weeks, piglets consumed 78% of the 2400/13.5 diet in the high-contrast pair, 70% of the 2344/15.5 diet in the moderate-contrast pair, and 60% of the 2316/16.5 diet in the low-contrast pair. Time spent at the feeder reflected feed intake patterns, whereas the number of feeder visits did not differ between diets within any pair throughout the trial. Despite these pronounced preferences, no differences in final body weight were observed after three weeks of dietary choice, although piglets offered the low-contrast diets were numerically heavier by approximately 1.6 kg compared with those in the other pens. Similarly, no differences—either statistical or numerical (<1.5%)—were detected in feed conversion ratio. In conclusion, when faced with strong dietary contrasts, piglets preferentially overconsume energy-rich diets relative to protein-rich diets, demonstrating a clear preference for high-energy feeds. Importantly, these dietary imbalances did not impair growth performance compared with piglets consuming a conventional complete diet.

Effects of incomplete milking during summer on metabolic and oxidative status in peak-lactation dairy cows: a pilot study

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Heat stress places a significant metabolic burden on high-producing dairy cows, as the energy required for milk synthesis increases endogenous heat production and limits thermoregulatory capacity under elevated ambient temperatures. Temporarily reducing mammary nutrient demand may therefore serve as a physiological strategy to lessen metabolic strain during thermal challenges. As a digital innovation, incomplete milking (IM) can be defined using a specialized software for a controlled reduction in milk withdrawal. Previously, it has been shown to decrease lactation-associated metabolic load without compromising udder health or subsequent productivity. The objective of this pilot study was to apply an established IM protocol during peak lactation under summer conditions and evaluate its effects on milk yield, metabolic and oxidative cell status. In summer 2024, clinically healthy cows in peak lactation were assigned to either complete milking (HCOMP; n=3; DIM 46±16; parity 2.3±0.3) or incomplete milking (HINCL; n=3; DIM 62±14; parity 2.3±0.3). In HINCL cows, milk withdrawal was reduced by 9% per day over 5.5 d to achieve an approximate 40% reduction (phase 1), followed by maintenance of this reduction for an additional 5.5 d (phase 2). One week prior to the intervention (baseline) and two weeks following the intervention served as reference periods. Milk yield and feed intake were recorded daily, and blood samples were collected on d -2, 6, 13, 20, and 27 relative to the start of IM for analysis of non-esterified fatty acids (NEFA), insulin-like growth factor-1 (IGF-1), derivatives of reactive oxygen metabolites (dROM), and advanced oxidation protein products (AOPP). Due to the limited sample size, results are presented descriptively. The average daily THI was 65±0.56 during phase 1, 63±0.89 during phase 2, and 70±0.46 in the week post-IM. Baseline milk yield ranged from 24.1 to 48.9 kg/d in HINCL and from 32.2 to 43.4 kg/d in HCOMP. In HINCL cows, the targeted reduction in milk withdrawal during the two IM phases was successfully achieved. During phase 1, NEFA concentrations were variable in both groups (HCOMP: 0.35–0.51 mmol/L; HINCL: 0.16–0.61 mmol/L). In phase 2, NEFA concentrations decreased and stabilized at lower levels (HCOMP: 0.11–0.18 mmol/L; HINCL: 0.10–0.12 mmol/L), then increased after cessation of IM. Baseline IGF-1 concentrations were comparable between groups (HCOMP: 81.1–126.8 ng/mL; HINCL: 97.9–138.9 ng/mL). While IGF-1 concentrations in both groups remained within baseline ranges during phase 1, HINCL cows showed a marked increase during phase 2 (166.9–303.0 ng/mL), whereas HCOMP cows showed only moderate variation (105.1–147.2 ng/mL). Baseline dROM values ranged from 42.4 to 104.9 µg H2O2 equivalent/mL in HINCL and from 54.9 to 121.9 µg H2O2 equivalent/mL in HCOMP. During phase 2, dROM values in HINCL cows increased markedly (111.0–138.8 µg H2O2 equivalent/mL), whereas HCOMP cows showed different trends by individual (93.6–116.9 µg H2O2 equivalent/mL). In contrast, AOPP values per g protein in HINCL decreased to a lower level in phase 2 (baseline: 0.97–1.18 µmol/g, phase 2: 0.74–1.03 µmol/g), whereas AOPP values in HCOMP were variable in both weeks (baseline: 0.63–1.19 µmol/g, phase 2: 0.95–1.32 µmol/g). Due to the limitations of this pilot study, it is not possible to provide a definitive answer to the targeted research question. Still, these preliminary observations support further investigation of IM as a potential strategy to modulate metabolic load during periods of thermal challenge in dairy cows.

Hoof Temperature Differences in Relation to Lameness Score

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Besides mastitis and reproductive problems, lameness is one of the most common issues on dairy farms. Lameness has a negative effect on dairy herd performance and farm profitability due to its association with reduced milk yield, an increased risk of culling, impaired reproductive performance and also has negative impact on the cows' welfare. Early detection of cows with a lower degree of lameness is therefore essential to prevent progression to more severe stages and to improve overall herd welfare. Combining different methods, particularly those with the potential for automation, could significantly improve heard health monitoring and farm management. In order to improve the detection of lame cows on the farm, the aim of this study was to monitor hoof temperature and its relationship with the lameness score. Infrared temperature values were recorded at the coronary band (CB) of the cow's hooves. The four-point manual locomotion scoring system was used to assess four levels of lameness score (LS0 – LS3). The coronary band temperatures of unhealthy hooves were significantly higher comparably compared to those of healthy hooves. Under ambient recording conditions with air temperature of 14.27°C and relative humidity of 85.57%, significant differences in CB temperature were observed between cows with different lameness scores. The IR MEAN values of cows with LS0 (16.28°C) were significantly ($p < 0.05$) lower than those of cows with LS1 (17.59°C), LS2 (17.71°C) and were highly significantly lower compared to cows with LS3 (18.04°C). The results obtained in this study further advance the application of thermography by demonstrating that coronary band temperature distribution is influenced by hoof health status, ambient recording conditions, and lameness severity. The combination of methods used in this study enables early, non-invasive monitoring of hoof health and supports practical implementation within precision livestock farming frameworks. Although the practical implementation of automated systems still presents certain challenges, the combination of different methods that increase the reliability and accuracy of hoof disease detection may improve the prevention of lameness under farm conditions in the future, although further development is still required.

Improving Real-Time Sow and Piglet Behavior Detection Using Mixed-ELAN Architecture

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The need for effective livestock management has increased with the evolving dynamics of the agricultural industry, characterized by an aging workforce and the expansion of large-scale farming operations. Conventional monitoring methods, which rely predominantly on manual observation, are increasingly reaching their limits, necessitating the development of innovative automated solutions. In this study, a system for real-time sow and piglet behavior detection using an extended version of the ELAN architecture, known as mixed-ELAN, using diverse kernel sizes was presented. The standard convolutional operations within the ELAN framework were replaced by MixConv using diverse kernel sizes to enable superior feature learning capabilities. The proposed structure was applied to YOLOv7 and YOLOv9, and the experiments showed improvements of 1.5% and 2%, respectively, with a mean average precision of 0.805 and 0.796, compared to the original models. In addition, both models demonstrated significant performance improvements in behaviors critical for piglet growth and survival, such as crushing and lying down, highlighting the effectiveness of the proposed architecture. These advances underscore not only the potential of leveraging AI and computer vision technologies in agricultural practices, but also the benefits of the system in enhancing animal welfare and farm management efficiency. The proposed architecture, by providing a more sophisticated comprehension and real-time monitoring of livestock behavior, establishes higher benchmarks for smart farming technologies, and opens up possibilities for further innovation in livestock management.

AI-Based Early Warning System for Tail Biting Risk Detection suitable for Diverse Pig Husbandry Systems

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Tail biting represents one of the most persistent welfare and economic challenges in intensive pig production systems, leading to pain, tissue damage, infection, reduced growth performance and increased antibiotic use. Effective prevention depends on the early detection of behavioral changes preceding visible injury. Previous scientific initiatives, including "DigiSchwein" and related precision livestock farming projects, have demonstrated the feasibility of automated monitoring in pig husbandry. However, a scalable real-time early warning system specifically addressing tail biting risk under diverse farm conditions remains insufficiently established. This research is conducted within the framework of the "DeTail – Detection of Tail Biting" project funded by the European Innovation Partnership Agrar (EIP-Agrar) in Schleswig-Holstein and led by Landwirtschaftskammer Schleswig-Holstein in cooperation with the University of Oldenburg and Christian-Albrechts-University Kiel as scientific partners. The project aims to bridge this gap by integrating deep learning-based tail posture detection with motion-derived behavioral threshold modelling. Pigs exhibiting hanging tails are more frequently associated with stress, discomfort or ongoing biting events. Building upon these findings, this study implements a deep learning object detection architecture based on the YOLO framework. The model is trained via transfer learning on heterogeneous annotated datasets from multiple farms to detect individual pigs and classify tail posture states (tail up, tail down) together with posture categories (pig lying, pig not lying). For the prototype implementation, 392 training images and 158 validation images from ten pens across four farms were used. Despite the relatively small yet environmentally diverse dataset, transfer learning from a pre-trained model enabled stable convergence and effective feature reuse. The resulting performance achieved an overall mAP@0.5 of 0.816 across all classes. Class-wise precision–recall results show strong performance for pig not lying (0.964) and pig lying (0.909), while tail up (0.656) and tail down (0.736) reflect the increased complexity associated with small-object detection and class imbalance. Given the limited number of annotated tail instances, transfer learning proved essential for reliable discrimination between tail posture states. The system processes live video streams directly within the barn and aggregates detections at per-second intervals to generate structured behavioral time series. By combining tail posture distributions with motion-based activity thresholds, adaptive alarm points indicating elevated tail biting risk are estimated. Unlike earlier monitoring systems that relied primarily on offline video analysis, DeTail implements real-time processing within the barn environment, eliminating continuous video transfer and reducing bandwidth requirements. Alarm states are visualized in a dashboard interface accessible to farm operators, supporting timely intervention and enabling objective and continuous behavioral assessment. This system advances precision livestock farming toward welfare-oriented and demonstrate how AI can transition from experimental research to field-ready system under EU animal welfare directives.

Dietary Phosphorus Restriction Alters IGF Availability Independent of Hepatic GHR Signalling in Young Goats

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Reducing dietary phosphorus (P) and nitrogen (N) intake in livestock is discussed as a strategy to decrease nutrient excretion and environmental pollution. Owing to their capacity to recycle phosphate (Pi) and to utilise N via microbes in the forestomach, ruminants are a particularly promising group for reducing the P and N content of feeds. However, both nutrients are tightly linked to endocrine growth regulation. Dietary N restriction is known to impair the somatotropic axis via downregulation of hepatic growth hormone receptor (GHR) expression and reduced circulating IGF1 in young goats [1]. Dietary P restriction has also been associated with lower plasma IGF1 in these animals [2], but the underlying mechanisms remain unclear. We therefore aimed to characterise the effects of reduced P intake, alone or combined with N restriction, on the hepatic GHR-IGF1 axis in young goats and hypothesised that reduced P intake impairs IGF availability through mechanisms analogous to N restriction. Twenty-eight young male goats were assigned to four feeding groups (adequate vs reduced P, with or without concurrent N reduction; n = 7 per group) in a six-week feeding trial. After slaughter, blood and liver samples were collected. Hepatic mRNA expression of components of the GHR-IGF system (including IGF1, IGF2 and acid-labile subunit (ALS)) was quantified, and plasma protein concentration of IGF binding proteins (IGFBP2 and IGFBP3) was assessed. Data were analysed using two-way ANOVA; statistical significance was set at $P < 0.05$. Reduced P intake decreased hepatic IGF1 ($P < 0.05$) and ALS ($P < 0.01$) mRNA expression. At protein level, P restriction reduced circulating IGFBP3 concentration ($P < 0.01$) and lowered both the IGF1:IGFBP3 ratio ($P < 0.05$) and the IGFBP3:IGFBP2 ratio ($P < 0.001$). In contrast, hepatic GHR expression and indicators of downstream JAK–STAT signalling were not affected by reduced P intake. Hepatic IGF2 mRNA expression was increased under P restriction ($P < 0.05$). In conclusion, dietary P restriction reduces circulating IGF1 independently of hepatic GHR/JAK-STAT signalling. The concomitant reduction in circulating IGFBP3 and hepatic ALS mRNA suggests altered IGF transport and/or availability, potentially reflecting changes in ternary complex formation or stability. These findings indicate that the endocrine response to P restriction in young goats differs fundamentally from that observed under N restriction and should be considered when developing reduced P feeding strategies to mitigate environmental P pollution. Acknowledgement: The authors gratefully acknowledge the financial support of the H.W. Schaumann Stiftung. References Firmenich, C.S., et al., Modulation of growth hormone receptor-insulin-like growth factor 1 axis by dietary protein in young ruminants. Br J Nutr, 2020. 123(6): p. 652–663.Zillinger, L.S., et al., Effects of dietary nitrogen and/or phosphorus reduction on mineral homeostasis and regulatory mechanisms in young goats. Frontiers in Veterinary Science, 2024. 11.

Maternal methionine supplementation in low-protein diets during late gestation and lactation increases the performance and alters fecal microbiota of the sows and their offspring

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Methionine (Met) is known to enhance antioxidant defense and reproductive efficiency in sows, but its role under low-protein feeding conditions is unclear. This study evaluated the effects of Met supplementation on lactation performance, antioxidant status, and gut microbiota of sows and their piglets. Sixty-six multiparous sows were assigned to a normal protein diet (18% crude protein, CP) or low-protein diets (15% CP) containing graded standardized ileal digestible Met levels from day 107 of gestation to day 21 of lactation. Compared with normal protein diet, low-protein diets supplemented with 0.30% Met significantly ($P < 0.05$) reduced serum urea nitrogen in sows at weaning and improved milk yield, piglet growth, and post-weaning estrus interval. In addition, 0.30% Met supplementation resulted in enhanced milk immunoglobulin M, glutathione, and taurine concentrations and increased serum antioxidant enzyme activities. Microbiome analysis indicated higher relative abundance of Christensenellaceae_R-7_group in piglets from the 0.30% Met group, positively correlated with growth traits. These findings suggest that a 0.30% Met level in a 15% CP diet supports sow performance and piglet development comparable to normal protein feeding through improved antioxidant capacity and beneficial microbial modulation.

Comparison between copper sources and benzoic acid supplementation in weaned piglets

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Monovalent copper has been reported to exhibit stronger antibacterial properties than divalent copper. Benzoic acid is commonly used in pig nutrition as a feed preservative and gut health additive and has been reported to form stable complexes with cuprous ions in aqueous environments, potentially stabilizing copper in its monovalent form. This study evaluated the effect of benzoic acid supplementation on the efficacy of dietary copper from monovalent (CR) or chelated divalent (MHA-Cu) sources in weaned piglets. A total of 1,200 piglets (Danbred × Pietrain), weaned at 24 days of age with an average body weight of 5.6 kg, were allocated to a 2 × 2 factorial design evaluating copper source (CR vs. MHA-Cu) and benzoic acid inclusion (0 or 0.5%). Four treatments were tested with 12 replicates of 25 pigs each. The trial lasted 42 days and consisted of three phases (1–14 d, 15–28 d, and 29–42 d). Copper was supplied at 140 ppm during phases 1 and 2 and 25 ppm during phase 3. Growth performance, mortality and morbidity, medication use, and fecal parameters (score and pH) were recorded. Data were analyzed using the General Linear Model procedure of JMP (version 17.0) with the pen serving as the experimental unit. During phase (Phase 2), pigs fed CR showed significantly higher feed intake compared with pigs fed MHA-Cu ($P < 0.05$). No significant differences in body weight were observed among treatments, although pigs fed CR with benzoic acid displayed numerically higher average daily gain and improved feed conversion ratio over the 42-day period compared with both MHA-Cu treatments. In Phase 1, pigs receiving CR showed the lowest proportion of individually medicated animals, indicating improved adaptation to weaning. Overall mortality and morbidity tended to be higher in pigs fed MHA-Cu ($P < 0.10$). During Phase 2, pigs receiving MHA-Cu alone exhibited higher fecal scores compared with pigs fed CR or MHA-Cu with benzoic acid, indicating reduced intestinal stability. A tendency for higher fecal pH was observed in pigs fed MHA-Cu with benzoic acid compared with CR and MHA-Cu alone, which may reflect reduced hindgut fermentation due to antimicrobial activity earlier in the digestive tract. No differences were detected in total fecal enterobacteria counts among treatments. Overall, CR supported higher feed intake during the critical starter phase and numerically improved growth efficiency, health status, and fecal consistency. Benzoic acid supplementation did not consistently enhance responses across copper sources, indicating that the primary performance benefit was driven by the copper source. These results demonstrate that monovalent copper is a reliable alternative to chelated copper sources in commercial weaned piglet diets.

Effect of trace mineral source on copper, manganese, and zinc concentrations in ruminal content and feces of crossbred steers in grazing conditions

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Copper, Mn, and Zn from highly soluble sources in the rumen, can strongly bind to fiber, reducing absorption in the small intestine. Thus, our objective was to evaluate the effect of different trace mineral sources on Cu, Mn, and Zn concentrations in the ruminal contents and feces of crossbred steers under grazing conditions. Four steers were assigned to a 4×4 Latin square design: Control (No supplemental Cu, Mn, or Zn); Cu, Mn, and Zn-sulfate; Cu, Mn, and Zn-organic; and Cu, Mn, and Zn-hydroxychloride. Samples of feces and ruminal contents were collected at the end of each period and analyzed for Cu, Mn, and Zn concentrations. Data were subjected to analysis of variance ($P < 0.05$), and the means were separate using the SNK multiple comparison test. No treatment x animal x period interactions were observed ($P > 0.05$). Fecal Cu concentrations were greater ($P < 0.05$) in animals receiving sulfate forms of Cu, Mn, and Zn compared to all other treatments. Fecal Cu concentrations were similar between hydroxychloride, organic, and control steers. Fecal Mn concentrations were greater ($P < 0.05$) in steers receiving Mn-sulfate compared to all other treatments. Fecal Mn concentrations were similar between steers receiving hydroxychloride and organic forms of Mn. Steers receiving Mn in sulfate, hydroxychloride, and organic forms had greater fecal Mn compared to controls. A similar pattern was observed for fecal Zn concentrations. Copper, Mn, and Zn concentrations in the solid fraction of the ruminal contents were greater ($P< 0.05$) for the steers receiving sulfate forms compared to all other treatments. Copper, Mn, and Zn concentrations in the solid fraction of the ruminal contents were similar between steers receiving hydroxychloride, organic, and control diets. Overall, sulfate sources increased the concentrations of Cu, Mn, and Zn in both feces and in the solid fraction of the ruminal contents, suggesting that Cu, Mn, and Zn from sulfate bind strongly to digesta.

Calcium, bone and energy metabolism of dairy goats supplemented with poplar leaves during early lactation

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Early lactating dairy goats experience increased energy and nutrient demands for colostrum and milk production, which cannot be met by a sufficient increase in feed intake. As a consequence, does mobilize, among others, their fat and bone reserves, thereby entering negative energy and Ca balances. Black poplar leaves are rich in metabolizable energy and Ca, suggesting they are a valuable feed source for meeting the increased requirements of early lactation. Therefore, the objective of this study was to investigate the effect of black poplar supplementation on the Ca, bone and energy metabolism of early lactating dairy goats. Sixteen one-year old Saanen goats in the third trimester of pregnancy were group housed at least four 4 weeks until parturition. Goats were fed hay ad libitum, wheat, 0.2 to 0.3 kg wheat daily and had access to a mineral lick stone. After birth, access to the mineral lick stone was blocked and dams and their lambs were moved to individual pens and assigned to one of two groups. One group was fed 700 g of black poplar hybrid foliage, gradual increase in wheat (100 to 250 g from week 1 to 5 postpartum), and hay for ad libitum intake (POPLAR, n = 9). The control group received only hay for ad libitum intake and wheat as described (CON; n = 7). Individual foliage and hay intakes were recorded daily and dams were blood sampled weekly starting 1 week before the calculated lambing date. Both groups had a comparable dry matter intake and plasma glucose and non-esterified fatty acid concentrations. However, POPLAR goats tended to have lower plasma osteocalcin concentrations postpartum and had a lower osteocalcin/CTX ratio and lower serum Ca concentrations in week 2 pp postpartum. These results indicate that black poplar supplementation had no impact on the energy status but compromised calcium and bone metabolism, the latter likely due to tannins hindering intestinal Ca absorption. Therefore, black poplar supplementation cannot be recommended as feed supplement in early lactation.

Discovering the plasma metabolic fingerprint of heat-stressed Holstein dairy cows

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Heat stress can impact dairy cow feeding behavior, ruminal function, intermediary metabolism and nutrient partitioning. Wholesale changes in plasma metabolic profiles may serve as a tool to provide further mechanistic insights in regards to the heat stress phenotype. This study aimed to elucidate the plasma metabolic profile differences between cows under heat stress or feed restriction in thermoneutrality. Thirty German Holstein cows (169 ± 9 days in milk; DIM) were kept in a climate chamber under thermoneutral conditions (16°C; 63% relative humidity (RH); temperature-humidity-index (THI) 60) for 6 days and fed a total mixed ration for ad libitum intake. Subsequently, 10 cows were exposed to 28°C (heat stress, HS; 52% RH; THI 76) for 6 days, whilst 10 cows remained under thermoneutral conditions (CON). To mimic the lower feed intake under HS, pair-feeding using a diet identical to the HS group was applied to the CON group. Milk yield, dry matter (DMI) and water intake were determined daily. Rectal temperature (RT) and respiration frequency (RF) were measured twice daily. Blood samples were collected on day 6 after treatment for targeted metabolomic analysis. Plasma samples were subjected to targeted quantitative metabolomics using reverse-phase HPLC/MS/MS. Statistical analysis of plasma metabolites was performed by utilizing MetaboAnalyst 6.0. Daily measurements on the same animal were analyzed with a repeated measure ANOVA using the MIXED procedure of SAS (V9.4) with the fixed effects of treatment (HS, CON, PF), time (day), and treatment by time interaction; and DIM served as a covariate. Multiple comparisons were performed with the Tukey-Kramer test. Milk yield was reduced by 25% in HS than CON cows, whilst in the PF group, milk yield decreased by 13% relative to the CON group ($P<0.05$, respectively). DMI was lower in HS and PF than CON cows, whereas water intake was higher in HS than PF and CON cows ($P<0.05$, respectively). RT and RF increased in HS than CON and PF cows ($P<0.01$, respectively). In total, 164 plasma metabolites were detected, of which 53 metabolite concentrations were differently abundant. HS cows exhibited higher plasma concentrations of creatinine, methylhistidine, acetylornithine, sphingomyelins SM (OH) C22:1, C22:2, SM C24:1 and methionine sulfoxide concentrations than CON and PF cows, whilst butyrylcarnitine concentration was significantly lower ($P<0.05$). Oleic acid, linoleic acid, margaric acid, palmitoleic acid, butyric acid, docosapentaenoic acid, heptanoic acid, methylmalonic acid myristic acid and pentadecylic acid concentrations were higher in PF than in HS and CON cows, whereas hippuric acid, alanine, uric acid, tyrosine, and propionic acid concentrations were lower in PF than in CON and HS cows (all $P<0.05$). To conclude, heat stress increased the concentrations of metabolites related to skeletal muscle proteolysis, oxidative protein modification and sphingolipid metabolism, independent of reduced feed intake. Plasma profile of cows with a reduced feed intake at thermoneutrality suggests increased fatty acid mobilization, altered rumen-derived metabolites, and enhanced phospholipid turnover.

Proteome characterization of milk extracellular vesicles isolated from cows during heat stress

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Heat stress (HS), intensified by climate change, threatens dairy cow welfare and productivity, yet early molecular adaptations remain unclear. Milk-derived extracellular vesicles (mEVs) reflect the physiological state of mammary cells and represent promising non-invasive biomarkers. This study evaluated the effects of acute HS on the mEV proteome in lactating Holstein and Brown Swiss cows. Fourteen multiparous cows (7 Holstein, 7 Brown Swiss) were exposed to thermoneutral (TN; THI < 68) and HS (THI 69–84) conditions by deactivating the cooling system in a commercial farm (July 2023). Cows were sampled under TN conditions and again after 24 h of HS exposure. Milk and blood samples were collected at 3 p.m. under both conditions. Systemic biomarkers included adiponectin, leptin, haptoglobin, and lactoferrin, along with oxidative stress indicators (d-ROM, FRAP, and AOPP). mEVs were isolated by ultracentrifugation and size-exclusion chromatography, characterized by nanoparticle tracking analysis and transmission electron microscopy, and analyzed by nano–LC–MS/MS. Proteomic data were identified by MASCOT and quantified by Progenesis QI using the Bos taurus UniProt database. The differential abundance of proteins was assessed using a univariate analysis with Benjamini-Hochberg correction, with HS as the main fixed effect. This was complemented by multi-level sparse partial least squares discriminant analysis(sPLS-DA), in which the proteins with non-zero loadings in the most discriminating HS component were retained and listed by using R software. Acute HS induced moderate physiological changes without affecting milk yield or most systemic biomarkers. However, significant remodeling of the mEV proteome was observed. Overall, 134 proteins were differentially abundant between TN and HS conditions (24 increased, 110 decreased under HS). Multivariate analysis showed clear separation between groups, with positive loadings under HS mainly driven by signal peptidase complex subunit 3, DnaJ heat shock protein family member C3, alpha-S2-casein, and keratin type II cytoskeletal 71. These proteins are associated with secretory activity, stress response, and structural organization. Functional enrichment indicated modulation of metabolic, immune and stress-response pathways. These results demonstrate that acute HS markedly alters mEV protein cargo, highlighting mEVs as sensitive indicators of early heat stress adaptation in dairy cows.

Heat stress induced alterations in the hepatic lipid metabolism in primiparous dairy cows

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Heat stress is a global challenge for dairy cows due to rising environmental temperatures. Heat stress has shown to induce metabolic adaptations, such as reduced lipolytic activity and nutrients prioritization, to cope with increased heat and concomitantly reduced feed intake. This study aims to investigate heat stress-induced alterations in the hepatic lipid metabolism in Holstein dairy cows. In this study, 30 mid-lactating, non-pregnant German Holstein dairy cows were randomly selected and acclimated for six days to thermoneutral conditions (TN) at 16°C, 63% relative humidity (RH), temperature-humidity-index (THI) of 60 in climate-controlled chambers. Thereafter, animals were evenly allocated to heat stress (HS), control (CON), or pair-feeding (PF) group (n = 10 cows per group) and subjected to a 7-day trial. HS group was exposed to 28°C, 52% RH and THI of 76, whereas CON and PF groups remained under TN. On trial day 7, cows were slaughtered and liver samples were collected. Gene expression related to lipid metabolism was quantified by RT-qPCR. Hepatic fat content and fatty acids (FA) profiles (percent of total FA) were determined using high-resolution gas chromatography. Statistical analyses were conducted using linear mixed models in R. HS cows showed a tendency towards lower percentages of total liver fat ($P<0.09$) and monounsaturated FA (MUFA) than PF cows, whereas PF cows had higher percentage of total fat and MUFA than CON cows ($P<0.001$, respectively). Saturated FA were comparable among the groups. Further, HS cows had higher percentage of n-3 PUFA than CON and PF cows and higher n-6 PUFA than PF cows ($P<0.001$, respectively). In total, 31 FA were detected, of which 25 FA were differently abundant and 6 FA were unaltered among the groups. HS cows tended to have lower C14:0 ($P<0.09$) and significantly lower C16:0 percentage than PF cows ($P<0.001$), but higher percentage of C17:0 and C18:0 than PF cows ($P<0.05$, respectively). In addition, HS cows had a higher percentage of C16:0 but lower C17:0, C21:0 and C23:0 percentages ($P<0.05$, respectively) and a tendency of a lower C18:0 portion ($P<0.09$) compared to CON cows. Percentages of C16:1cis-9, C17:1cis-9, C18:1cis-9, C18:1trans-9 and C18:1trans-11 were lower in HS than PF cows ($P<0.05$, respectively). A lower proportion of C18:1trans-9 but higher C18:1trans-11 was found in HS than CON cows ($P < 0.001$, respectively). The PUFA fraction had higher C18:2cis-9trans-11 and C20:3n-3 and lower C18:3n-3, C18:4n-3, C20:3n-6 and C20:5n-3 (EPA) percentages in HS than CON cows ($P<0.05$, respectively). Percentage of C20:3n-3, C20:3n-6, C20:2n-6, C20:4n-6, and C20:5n-3 tended or were significantly higher in HS than PF group ($P<0.09$, respectively). The mRNA abundance of hepatic sterol-CoA desaturase (SCD1) was lower in HS and PF than in CON cows ($P<0.001$, respectively). Similarly, mRNA abundance of acetyl-CoA carboxylase (ACACA) tended to be lower in HS than in CON cows ($P<0.09$), whilst mRNA abundances of fatty acid synthase (FASN) and ACACA tended ($P<0.09$) or were lower ($P<0.05$) in the liver of PF than CON cows. In conclusion, differences in hepatic fatty acid composition between the HS and the other two groups are likely due to differences in the mobilization of extrahepatic fat and the concomitant suppression of de novo lipogenesis in HS and PF cows. This suggests that the amount and profile of circulating fatty acids may have influenced hepatic lipid composition in the liver of HS and ectopic fat storage in the liver of PF cows.

Predicting subclinical ketosis in Holstein dairy cows using fatty acid content in milk

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Subclinical ketosis (SCK) is a metabolic disorder in dairy cows that occurs during the early postpartum period and is associated with reduced feed intake, decreased milk yield, increased risk of clinical ketosis, displaced abomasum, and impaired fertility. It is characterized by elevated levels of ketone bodies in the blood, milk, and urine. A blood serum level of β-hydroxybutyric acid (BHBA) of 1.2 mmol/l is proposed as a suitable threshold value for the initial detection and treatment of SCK. The milk fat content and fatty acid (FA) profile in particular, are promising indicators of negative energy balance during early stages of lactation. The main objective of this study was to evaluate whether specific milk FA profiles can provide precise indicators for identifying cows at risk of SCK, using their association with BHBA as a diagnostic reference parameter. The data was collected on ten dairy farms in Germany. On each farm, ten Holstein dairy cows were selected for the study. The primary criterion for selection was the nearest anticipated calving date (2–3 weeks) in relation to the initiation of data collection. The data collection process was conducted over a period of 11 days per farm. BHBA was determined on days 2, 4, 9 and 11 after milking using blood samples. The FA profile was determined using mid-infrared spectra-based prediction equations. The quantities of FA were calculated as the proportion of milk fat. Milk fat originates from the synthesis of FA in the mammary gland, from FA supplied by the diet or from FA released from body fat during a negative energy balance. The following FA were selected for the ensuing analysis: C4:0, C6:0, C8:0, C10:0, C12:0, C14:0, C14:1, C15:0, C15:0 anteiso, C15:0 iso, C16:0, C16:1 cis, C17:0, C18:0, C18:1 cis-9, C18:2 cis-9 cis-12, C18:2 cis-9 trans-11, C18:3 cis-9 cis-12 cis-15, and total C18:1 trans. Due to multicollinearity among the different FA, they were clustered (CL) into 9 groups of correlated milk FA by cluster analysis. A binary parameter (0 = SCK, 1 = no SCK) was created and used as the response variable in relation to the FA within each cluster. The statistical analysis to predict the occurrence of SCK was performed using a generalised linear model with a logit link function, considering parity as a fixed effect and FA as a regression term. The results of the study revealed a significant negative association between the concentrations of C14:0 ($P<0.0001$) and C8:0 ($P=0.0004$) (CL4) and the occurrence of SCK. In contrast, C4:0 (CL5) exhibited a significant positive ($P=0.008$) association with SCK, despite the fact that the FA in both CLs are predominantly synthesised in the mammary gland. A positive association was found between C18:0 (CL7) ($P<0.0001$), C18:1 cis-9 ($P<0.0001$) (CL9) and an increased risk of SCK. These long-chain FA are primarily derived from the mobilisation of body fat reserves. Furthermore, a negative association was found between C15:0 ($P<0.0001$) (CL1), an odd chain FA produced mainly by microbial fermentation, and the risk of SCK. The correlation between the FA mentioned above and BHB ranged from -0.5 to 0.34. This study shows that specific milk FAs are strongly linked to the risk of SCK in Holstein dairy cows and can serve as non-invasive indicators for its early detection.

Can milk fatty acid profile serve as a predictor of different types of hyperketolactia in dairy cows?

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This study aimed to explore relationships between selected fatty acids (FA) in milk and concentrations of beta-hydroxybutyric acid (mBHB) and acetone (mACE), and to develop models predicting the risk of hyperketolactia (HYKL) types for potential use in milk recording in Poland. Data from 1,023,838 test-day samples provided by the Polish Federation of Cattle Breeders and Dairy Farmers (PFHBiPM) between 10 August 2017 and 28 December 2019 were analyzed. Samples were collected from Holstein–Friesian cows at 6–60 DIM. Relationships between milk components were assessed using Spearman's rank correlations. Samples were classified into healthy (NKL) and hyperketolactic (HYKL) groups based on thresholds (de Roos et al., 2007): NKL (mACE < 0.15 mmol/L and mBHB < 0.1 mmol/L) and HYKL (mACE ≥ 0.15 mmol/L or mBHB ≥ 0.1 mmol/L; Kowalski et al., 2021). In addition, HYKL milk samples were further classified into three HYKL groups: HYKLACE (mACE ≥ 0.15 mmol/L and mBHB < 0.1 mmol/L), HYKLBHB (mACE < 0.15 mmol/L and mBHB ≥ 0.1 mmol/L), and HYKLACEBHB (mACE ≥ 0.15 mmol/L and mBHB ≥ 0.1 mmol/L). Multiclass logistic regression models were developed using DIM and selected milk traits (mBHB, mACE, MF, MP, LCFA, SCFA, MUFA, C16:0, C14:0, C18:0, C18:1) as predictors and HYKL groups as outcomes. Principal component analysis (PCA) was used to select key predictors. A strong positive relationship was found between mACE and mBHB (r = 0.78). Negative correlations were observed between mACE and the contents of SCFA and C14:0 (r = –0.26 and –0.23, respectively). The content of MP was weakly negatively correlated with both mACE and mBHB (r = –0.16 and –0.21). The strongest associations were observed between mACE and LCFA and TUFA (r = 0.45 and 0.43), as well as between mACE and C18:1 and C18:0 (r = 0.45 and 0.44). Similar relationships were found for mBHB (r = 0.49 for LCFA, r = 0.44 for TUFA, r = 0.46 for C18:1, and r = 0.48 for C18:0). The correlations observed between mBHB, mACE, and FA contents provide a foundation for further research on the use of these parameters in diagnostic models for monitoring dairy cow health. The most important predictors of assignment to particular HYKL groups were the ketone bodies, i.e., mACE and mBHB. The contents of C18:0 and C18:1 were also relatively strong predictors. In predicting HYKLACE, the most important variables were MUFA, TUFA, LCFA, C18:1, C18:0, MY, MP, C14:0, and DIM. In predicting HYKLBHB, the most important variables were mBHB, MY, MP, C14:0, C16:0, MF, C18:0, C18:1, and LCFA. In contrast, in predicting HYKLACEBHB, the most important variables were mBHB, mACE, DIM, SCFA, and C14:0.The developed models showed the lowest predictive accuracy for the HYKLACEBHB group, indicating greater complexity in correctly classifying cases where both ketone bodies are elevated. Overall, the results confirm that milk fatty acid profiles, particularly LCFA, MUFA, SCFA, C18:0, and C18:1, are significantly associated with ketone body concentrations and can be used as indicators in predictive models. Inclusion of selected FA improves the ability to differentiate between specific types of hyperketolactia.

Associations between hyperketonaemia, glucose and phosphate

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Hyperketonaemia (HK) develops when acetyl-CoA, mainly derived from non-esterified fatty acid (NEFA) oxidation, cannot be further metabolised in the citric acid cycle. In the present study, 48 cows from two dairy herds (Holstein-Friesian and Simmental) were monitored due to a known increased risk of clinical ketosis and associated health issues. Starting with day 1, animals were blood sampled weekly until 32 days in milk (DIM) and treated with oral propylene glycol for five days when serum β-hydroxybutyrate (BHB), determined using a hand-held device, exceeded 1.2 mM, and additionally with intravenous glucose monohydrate and fructose once when BHB exceeded 1.7 mM. If animals were treated intravenously, they were sampled again after 24 h and received a second i.v. treatment if BHB was still > 1.7 mM. During the observation period, 16 out of the 48 cows presented with HK and were treated either orally or intravenously once (HK1). Another eight HK cows were treated between three and twelve times (HK2). To gain further insight into intermediary metabolism and to evaluate whether animals at risk could be identified earlier, we analysed serum for NEFA, glucose, calcium and phosphate using standard methods conducted at the Large Animal Clinic Laboratory. Group differences were analysed using a mixed model (time, grade and interaction) followed by Tukey´s multiple comparisons test (grade). First test-day milk yield was higher in HK2 animals compared to non-ketonaemic cows (NHK) but did not differ from HK1 (HK2 vs. NHK: $P < 0.001$). On the second test-day, milk yield in HK2 also differed from HK1 (HK2 vs. NHK: $P = 0.003$; HK2 vs. HK1: $P = 0.029$). The body condition score of HK2 cows was lower compared to the other two groups at 16 DIM (HK2 vs. NHK: $P = 0.031$; HK2 vs. HK1: $P = 0.040$), 24 DIM (HK2 vs. NHK: $P = 0.008$; HK2 vs. HK1: $P = 0.016$), and 32 DIM (HK2 vs. NHK: $P = 0.032$; HK2 vs. HK1: $P = 0.042$). Serum NEFA and BHB were increased in both HK groups compared to NHK during the entire observation period except for 1 DIM, while a significant difference between HK1 and HK2 could only be detected for NEFA on 8 DIM ($P = 0.008$) and 16 DIM ($P = 0.030$), but not for BHB. Calcium concentrations did not differ between the groups, while glucose was deceased in HK1 cows compared to NHK. The most notable finding in HK2 animals was a significantly lower product of serum glucose and phosphate concentrations in comparison to both NHK and HK1 cows on 8 DIM (HK2 vs. NHK: $P < 0.001$; HK2 vs. HK1: $P = 0.001$) and 16 DIM (HK2 vs. NHK: $P = 0.003$; HK2 vs. HK1: $P = 0.014$) and compared to NHK on 24 DIM ($P = 0.035$) and 32 DIM ($P = 0.042$). When expressed relative to NEFA, a significant reduction compared to the NHK and HK2 groups was already evident on 1 DIM (HK2 vs. NHK: $P = 0.005$; HK2 vs. HK1: $P = 0.006$) and persisted until 24 DIM. This observation might indicate that the combination of these parameters could be associated with metabolic alterations linked to high milk yield; adjustments that might also explain the unresponsiveness of serum BHB to treatment in HK2. Such homeorhetic adjustments, however, may be potential risk factors for morbidity. Whether the product of glucose and phosphate related to NEFA on 1 DIM might be suitable for the early identification of cows that require special attention and possibly support would need to be investigated in a larger sample.

The association of postpartum inflammatory-metabolic status with early-lactation milk yield in clinically healthy cows

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In the early postpartum, most dairy cows experience some systemic inflammation from processes such as tissue injury and remodeling, microbial exposure in the uterus, mammary gland, and gastrointestinal tract, lipid mobilization, and oxidative stress. Although controlled inflammation is normal in the periparturient period, excessive inflammation is associated with reduced milk yield. Concurrently, decreased dry matter intake and increased energy and nutrient demands for milk production predispose cows to metabolic disorders such as subclinical hypocalcemia (SCH) and hyperketonemia (HYK), which are also associated with lower production. However, it is unclear whether the combination of excessive inflammation and metabolic disorders affects milk yield differently than either condition alone. We hypothesized that cows with both excessive inflammation and ≥1 metabolic disorder will have lower milk yield than cows with one or neither condition. Our objective was to evaluate the association of inflammatory-metabolic status with milk yield in early lactation (SCH and HYK were combined to represent overall metabolic dysfunction). Blood samples were collected from 513 Holstein cows from 2 herds at 1, 2, 3, 5, and 7 days in milk (DIM) to measure plasma haptoglobin (Hp), total calcium (Ca), and blood β-hydroxybutyrate (BHB). Only cows without metritis, clinical mastitis, milk fever, clinical ketosis, or displaced abomasum within 21 DIM were included (final n = 428). Excessive inflammation was defined as Hp above threshold on ≥2 days using parity- and DIM-specific thresholds from our previous study (primiparous: 0.4, 0.4, and 0.6 g/L at 3, 5, and 7 DIM; multiparous: 0.2, 0.3, 0.5, and 0.1 g/L at 1, 3, 5, and 7 DIM). Subclinical hypocalcemia was defined as total Ca ≤2.15 mmol/L at 2 DIM in primiparous cows and ≤2.20 mmol/L at 3 and/or 5 DIM in multiparous cows (McArt and Neves (2020); as 4 DIM was not sampled in our study, we applied multiparous threshold to the closest time points), and HYK as BHB ≥1.2 mmol/L at 3, 5, and/or 7 DIM. Cows were categorized into four inflammatory-metabolic groups: NON (no excessive inflammation, SCH, or HYK), MET (SCH and/or HYK only), INF (excessive inflammation only), and INF+MET (excessive inflammation with SCH and/or HYK). Repeated measures linear mixed models were used to assess associations between inflammatory-metabolic status and milk yield through wk 12 of lactation. In multiparous cows, INF and INF+MET cows produced 4-9 kg/d less than NON and MET cows during the first 4 wk of lactation, and INF+MET cows produced 5 kg/d less than INF cows in wk 1. In primiparous cows, INF cows produced 6-8 kg/d less milk than MET cows in wk 1 and 3 of lactation and 6–8 kg/d less than INF+MET cows from wk 4 to 12, whereas INF+MET cows tended to produce less than MET cows only in wk 1. Overall, excessive inflammation was associated with lower milk yield in multiparous cows regardless of metabolic status (NON & MET > INF & INF+MET). In primiparous cows, inflammation was associated with lower milk yield in the first wk regardless of metabolic status (NON & MET > INF & INF+MET); however, from wk 2 onward, cows with both inflammation and metabolic disorders showed production recovery, whereas cows with inflammation alone had decreased milk yield throughout the 12 weeks (NON & MET & INF+MET > INF). These findings suggest that parity modifies the association between inflammatory–metabolic status and early-lactation milk yield, with inflammation appearing to be the primary factor associated with reduced production in multiparous cows, whereas concurrent metabolic disorders may alter this relationship in primiparous cows.

Effect of phosphate source on phosphorus digestibility in cows

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Phosphorus (P) is an essential mineral for the production and health of dairy cows. As basic diets do not always meet nutritional requirements, an inorganic phosphate (Pi) supplement may be necessary to ensure adequate P intake. However, P digestibility (dP) may vary among Pi sources and these variations remain poorly described. To clarify the dP values of various products used in cow nutrition, a digestibility study was conducted. At the same time, the impact of different forms of Pi on P metabolism was investigated, as well as the impact on the total digestibility of other dietary components. Twelve Prim'Holstein cows (560±68 kg, 5.5±1.7 yo) were divided into six homogeneous groups of two animals and assigned to a 6×6 Latin Square (with 7-day periods). The diet, individually monitored, was formulated to meet 100% of individual energy and protein requirements, but only 75% of P requirements. To meet 100% of theoretical P requirements, additional P was provided with one of the 5 following Pi supplements: dicalcium phosphate (DCP), monocalcium phosphate (MCP), monoammonium phosphate (MAP), monosodium phosphate (MSP), magnesium phosphate (MagP). During one of the experimental period of the Latin Square, no supplement was provided (control treatment). Feces were collected and analyzed during the last three days of each experimental period. Urine, blood, and saliva samples were also collected for P measurement on the last day of each experimental period. True dP (dPDiet) was calculated using an indigestible marker (TiO_2) to estimate fecal output, and endogenous P losses were predicted from Meschy (2003). Phosphate dP (dPPi) was obtained by difference, assuming identical basal dPDiet as in the control treatment. The effect of Pi source was tested using a GLIMMIX procedure (SAS) and LSmeans were compared using the Tukey-Kramer method. The substantial differences in dPDiet and dPPi between treatments were not significant (Table 1) due to the high inter-individual variability. DCP increased urinary P concentration, suggesting a modification in P metabolism. Numerically, dPPi was lower with MCP, with no notable difference in the digestibility of the other components of the ration or in the P concentration in the matrices analyzed. The MagP treatment stood out with numerically higher digestibility of NDF (P=0.147, +3.1%, in MagP compared to other treatments) and crude protein (P=0.163, +2.3%), suggesting an overall improvement in digestive efficiency. For this treatment, as the metabolic parameters of P do not stand out particularly compared to the other treatments, the effect might originate from ruminal ecosystem. This work has drawn references value for dP from different Pi sources. Except for MCP whose low dPPi was not explained, the dPPi of other supplements were ranked as expected in the literature (Jongbloed et al., 2002). In this study, Pi source had limited impact on dPPi but the trends observed in the digestibility of other constituents and P metabolism deserve closer examination.

Table 1. Digestibility and metabolism of P depending on Pi source.

	DCP	MCP	MAP	MSP	MagP	SEM	Treatment effect P-value
Digestibility of P							
dPDiet (%)	67.5	61.9	67.8	68.9	68.2	2.8	0.168
dPPi (%)	43.3	9.0	48.4	50.4	45.4	17.2	0.214
dPPi (% dPDCP)	100	21	112	116	105		
P concentration in matrices (mmol/L)							
Blood	1.69	1.67	1.70	1.64	1.72	0.06	0.795
Saliva	6.71	7.33	6.94	6.79	6.23	0.85	0.793
Urine	0.483a	0.231b	0.212b	0.226b	0.240ab	0.079	0.032

Changes in rumen fermentation pattern with additional dietary potassium supply

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Dietary cation-anion difference (DCAD) is calculated as the difference in concentration between sodium and potassium (K) cations and chlorine and sulfur anions. The beneficial effect of increasing DCAD or the electrolyte balance (EB calculated as DCAD minus sulfur) of a diet on performance of lactating dairy cows has been reported in several meta-analyses, which all agreed that dry matter intake (DMI), milk production and fat-corrected milk (FCM) are maximized at an EB level between +350 and +550 mEq/kg DM. Behind this performance response, changes in rumen fermentation pattern are among the main mechanisms, though these effects have been less studied. Therefore, the objective of the present work was to assess the effect of an elevated dietary DCAD level through additional K on the rumen fermentation of lactating dairy cows. Thirty multiparous Holstein cows from a commercial herd in China were splitted into two homogeneous groups based on initial milk yield (38.8 ± 1.20 kg), days in milk (DIM: 126 ± 33 days) and body weight (BW: 600 ± 25 kg). For 84 days, cows were fed either a typical corn silage-based diet alone (Control; 16.2% CP, 28.4% NDF, and EB level of 355 mEq/kg) or supplemented with 250 g/d of potassium carbonate hydrate (PCH; K-pron®, Evonik, 56% K on a DM basis; EB level of 508 mEq/kg). Individual DMI and milk yield were recorded daily, whereas individual milk composition was tested every 2 weeks. Intake was measured with a Roughage Intake Control (RIC) system (Hokofarm, NL). After 0, 42 and 84 experimental days, blood and rumen samples were taken on 6 cows, 2h after feeding. Blood was taken from the tail vein and analyzed for K concentration. Rumen samples were taken by stomach tubing, and analyzed for pH as well as for K, ammonia (NH3) and volatile fatty acids (VFA) concentrations. Data were statistically analyzed with R software according to a linear model with the fixed effects of treatment, sampling time and their interaction. Significance was declared at $P < 0.05$. Increased levels of K were observed in the blood and milk of cows fed PCH, highlighting the high bioavailability of the tested potassium source. Cows fed PCH presented higher rumen pH compared to control cows (6.48 versus 6.39; $P = 0.002$). Ammonia and total VFA concentrations were not different between diets (9.14 mM and 109 mM, respectively), but changes in individual VFA proportion were noticed. Rumen acetate proportion was higher with PCH (51.2 versus 49.6%; $P = 0.012$) leading to a higher acetate to propionate ratio (1.66 versus 1.49; $P = 0.001$). These changes in rumen fermentation pattern, most likely related to the rumen buffering effect of PCH (carbonate fraction), must have triggered the higher milk fat percentage observed for cows fed PCH (4.06 versus 3.90%; $P = 0.002$). As a consequence, FCM also tended to increase with PCH (+1.5 kg/d; $P = 0.061$). Other performance-related parameters such as DMI, milk yield, milk protein or milk urea were similar between groups. In summary, this study linked the beneficial effect of increasing the dietary DCAD level on performance of lactating dairy cows, with changes in rumen fermentation pattern.

The effects of total dietary copper, sulfur, molybdenum, and Cu:Mo ratio on Cu status of sheep: A meta-analysis

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Copper is an essential micronutrient for all ruminants, but sheep are especially susceptible to dietary imbalances due to poor metabolic regulatory mechanisms and antagonism between dietary Cu, S, and Mo which may reduce Cu absorption. Understanding what impacts plasma Cu concentration, the pool of Cu that is functionally available, may aid in developing nutritional management strategies. Therefore, a systematic literature review was conducted and data from 22 studies published between 1975 and January 2025 were compiled to evaluate the impact of varying dietary Cu, S, Mo, and the Cu:Mo ratio on plasma Cu concentration in sheep utilizing a meta-analytical approach. Data extracted from each publication included study design, study duration (d), number of experimental units including breed, sex, and age of sheep, dietary content of Cu, Mo, and S as well as form of Cu, Mo, and S when supplemented, end-of-study plasma Cu concentration, and end-of-study liver Cu concentration. Information on dry matter intake, average daily gain, and gain:feed were also extracted but due to a lack of reporting or incomplete information were not considered for use in modelling. All statistical procedures were conducted using SAS version 9.4 (SAS Institute Inc., Cary, NC). Prior to model development, the dependent variable (plasma Cu) was evaluated for normality using PROC UNIVARIATE. Mixed models were developed including the random effect of experiment with plasma Cu (mg/L) as the dependent variable. Fixed effects were evaluated for collinearity (and avoided), and effects were retained in models when $P \leq 0.05$. Independent variables assessed included the linear and quadratic effects of total dietary Cu and Mo (mg/kg DM), the Cu:Mo ratio (log transformed), total dietary S (% DM), end-of-study liver Cu (mg/kg DM), and study duration (d). Only independent variables with significance ($P \leq 0.05$) in univariable models were considered for inclusion in multivariable models. To account for heterogeneity in error and sample size across experiments, models were weighted by the inverse variance of the observation mean using the WEIGHT statement in PROC MIXED. Outliers were identified using the INFLUENCE option in PROC MIXED. Observations with a Cook's distance value greater than 4/n (where n = number of observations) were removed sequentially from the database. The final dataset included 22 experiments from 21 publications encompassing a total of 93 treatment mean observations. The final predictive models developed were evaluated using the developmental database. The model that best predicted plasma Cu included the quadratic effect of dietary Cu ($P = 0.044$), negative linear effect of dietary S ($P < 0.001$), and positive linear effect of dietary Mo ($P < 0.001$). The regression equation was expressed as: plasma Cu (mg/L) = 0.9761 (± 0.09280) + 0.0111 (± 0.00474) × Cu − 0.00012 (± 0.000056) × Cu^2 + 0.0055 (± 0.00110) × Mo − 0.6259 (± 0.17900) × S. This model demonstrated the best predictive performance with a concordance correlation coefficient (CCC) value of 0.835, a Pearson correlation coefficient (R) value of 0.850, and a root mean square prediction error (RMSPE) value of 14.11. No relation to study duration or liver Cu were observed ($P > 0.05$). Results support that dietary content of Cu, Mo, and S have significant influence on plasma Cu concentration of sheep. The positive effect of dietary Mo on plasma Cu concentration should be interpreted with caution as it does not indicate the form or availability of the Cu.

Influence of copper source on hepatic Cu accumulation, redox status and bile acid profiles in weanling and growing pigs

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Copper (Cu) is an essential trace mineral involved in multiple physiological processes. Although pharmacological Cu supplementation improves growth performance in pigs, high dietary inclusion has been associated with excessive hepatic accumulation, oxidative stress and alterations in lipid and glucose metabolism (1). The present experiment was conducted to test the hypothesis that use of Cu2O rather than CuSO4 in diets for weanling and growing pigs favourably affects post-absorptive tissue Cu distribution, hepatic oxidative status, and bile acid (BA) metabolism. A total of 120 weaned pigs (7.13 ± 0.6 kg) were randomly allocated to 3 dietary treatments. The treatments consisted in a negative control diet (NC), formulated to provide 6-7 Cu mg/kg of Cu in accordance with NRC requirements (2012), and two diets supplemented with 250 mg/kg of Cu, provided either as CuSO4 or Cu2O. There was a total of 8 pens/treatment, with 5 pigs/pen. Body weight (BW) and feed intake were periodically recorded. On the last day of the experiment (Day 84), one pig per pen was euthanized to obtain liver, bile, and colon samples. Cu concentrations in liver and bile were quantified by ICP-MS. Hepatic oxidative status was assessed using the thiobarbituric acid reactive substances (TBARS) assay and glutathione (GSH) determination. BA profiles were analysed in bile, liver and colonic mucosa by HPLC-MS/MS. One-way ANOVA was used to determine the variance among treatments, followed by Tukey's post hoc test at a significance level of $P < 0.05$. Pigs from Cu supplemented groups showed higher BW at the end of the study ($P < 0.05$) and increased hepatic and biliary Cu concentrations ($P < 0.001$) compared with the NC. Cu hepatic accumulation was higher ($P < 0.05$) in the CuSO4 group. Hepatic GSH was increased ($P < 0.05$) in the Cu2O group but not in the CuSO4 group. TBARS did not significantly differ among groups ($P > 0.1$). Both Cu-supplemented groups had altered BA profiles relative to the NC. Lower ($P < 0.05$) concentrations of primary BA (CDCA and HCA) were observed in the colon and liver of the Cu2O group. The secondary BA (HDCA, LCA) were reduced in the colon ($P < 0.01$) in both Cu-supplemented groups compared with the NC. Performance results and liver Cu concentrations observed in this experiment are consistent with previous studies (2). The differences in hepatic Cu concentrations may be attributed to the greater solubility of CuSO4 relative to Cu2O, leading in a greater intestinal absorption and hepatic accumulation. Oxidative stress marker results suggest a more effective antioxidant response in the Cu2O group compared with CuSO4. The BA profile observed in Cu2O group suggests reduced hepatic BA synthesis. Although the underlying mechanisms of BA modulation remain unclear, the results point towards a potential involvement of the FXR receptor and increased ileal reabsorption, in response to altered signalling. In conclusion, dietary Cu supplementation at 250 mg/kg in diets for weaned and growing pigs influenced hepatic Cu metabolism, redox status, and BA metabolism. Compared with CuSO□, Cu□O resulted in less hepatic Cu accumulation and a more effective antioxidant response, together with changes suggestive of reduced hepatic BA synthesis at the same inclusion level. Forouzandeh et al. (2022). Journal of Animal Science. Blavi, L., et al. (2021). Journal of Animal Science.

Genetics of traits derived from measures of milk electrical conductivity collected by milking robots and their correlations with clinical mastitis in Holstein cattle

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Genetic selection for mastitis resistance in dairy cattle has been conducted through both direct selection on clinical mastitis and indirect selection via correlated traits like SCS. Even with these efforts, mastitis remains a major concern for the dairy industry. Automatic milking systems collect measures of milk electrical conductivity (COND) for individual quarters of the udder at every milking visit, and traits derived from these measures may be good candidates for inclusion in selection indexes as indirect indicators of mastitis resistance. Therefore, the objectives of this study were to 1) derive multiple traits from quarter-level measures of milk COND collected by automatic milking systems; 2) estimate genetic parameters for traits derived from these measures; and 3) estimate (co)variance components between traits derived from measures of milk COND, health traits, and test-day traits in a population of American Holstein cattle. Quarter-level milk COND and visit milk yield were collected at every milking visit on 7,157 Holstein cows milked with automatic milking systems on a farm in north-central Indiana, USA. Additionally, producer-recorded events of clinical ketosis and clinical mastitis and test-day records of fat content, protein content, and SCS were available for an expanded population of cows that were milked in both automatic milking systems and conventional parlors. Eight traits were derived from quarter-level measures of milk COND collected at each milking visit. These visit-level traits were then summarized into daily values, either as the daily average or daily maximum, for subsequent analyses. Two additional traits were derived as the coefficient of variation or variance of all quarter-level measures of milk COND for an individual within a day. Additionally, data were analyzed separately by lactation: 1, 2, or 3+. Pedigree data for 53,186 individuals and genotype data for 29,126 individuals, imputed to 50,886 SNP, were used in the analyses. (Co)variance components were estimated using single-step GBLUP and linear animal models with REML. Estimates of heritability for traits derived from measures of milk COND ranged from 0.01±0.00 (range of COND scores; lactation 3+) to 0.52±0.01 (average of electrical COND; lactation 1). Estimates of genetic correlation between traits derived from measures of milk COND and clinical mastitis ranged from 0.27±0.09 (right-front COND score; lactation 3+) to 0.89±0.05 (coefficient of variation of COND scores; lactation 2), while estimates of genetic correlation between traits derived from measures of milk COND and SCS ranged from -0.05±0.09 (quarter extremes ratio of COND scores; lactation 1) to 0.76±0.03 (variance of COND scores; lactation 3+). Our results show that traits derived from measures of milk COND are heritable, and they have moderate to high genetic correlations with clinical mastitis, while having near-zero to high genetic correlations with SCS. These results suggest that traits derived from measures of milk COND are promising indicators of clinical mastitis and incorporating them into selection indexes in complement to traits already included in indexes for udder health can enhance genetic selection for mastitis resistance.

Personality-informed genomic prediction of sensor-derived feeding behaviour and resilience traits in dairy cows

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Large amounts of data from automated feeding systems enable the derivation of novel phenotypes capturing feeding behaviour and resilience in dairy cattle. In addition to environmental and physiological drivers, stable individual differences in personality may influence how cows interact with feeding resources, potentially leading to consistent differences in feeding behaviour and resilience. Therefore, this study aimed to (i) derive feeding behaviour and resilience traits from automated feeding records, (ii) estimate genomic parameters, and (iii) evaluate whether personality information improves their genomic prediction. Daily records from an automated feeding system (155,843 cow-days) were quality-controlled and restricted to days in milk (DIM) 5–400. Implausible observations were set to missing on a variable-specific basis, and short data gaps were interpolated within cow and lactation. Feeding behaviour traits were defined as feed intake per visit (IPV) and the proportion of unsuccessful visits (UVR). Resilience was quantified as the log-transformed variance of deviations from generalized additive model (GAM)–derived lactation trajectories (y ~ s(DIM)) for milk yield, dry matter intake, and UVR. Personality was assessed using standardized behavioural tests, including novel object and forced human approach tests. Responses were pre-adjusted for environmental effects and summarized into four personality dimensions using principal component analysis. The final dataset comprised 597 lactations from 263 cows with complete phenotype, personality, and genotype information. To improve the stability of individual-level estimates, cow-level phenotypes were derived as the median across available lactations. Additionally, personality traits were reduced to the first principal component (PC1; 30.1% variance explained), providing a composite personality descriptor. To estimate genomic parameters, a genomic relationship matrix based on 45,613 markers was used in genomic mixed models, with mean parity per cow included as a covariate to account for differences in lactation structure and information content. Genomic prediction was evaluated using repeated five-fold cross-validation with GBLUP. For this analysis, three representative traits were selected to illustrate prediction performance, and the potential contribution of personality information was assessed through changes in test-set RMSE and R^2. Sensor-derived feeding behaviour and resilience traits showed moderate to high genomic heritabilities (±SE), including IPV (0.68 ± 0.09), dry matter intake resilience (0.52 ± 0.10), UVR resilience (0.64 ± 0.09), milk yield resilience (0.42 ± 0.10), and mean UVR (0.32 ± 0.11). Genomic prediction accuracies, expressed as correlations between predicted and observed phenotypes, were 0.36, 0.38, and 0.48 for intake resilience, UVR resilience, and IPV, respectively. However, including personality PC1 did not improve prediction performance ($\Delta RMSE \approx 0$; $\Delta R^2 \approx 0$). Overall, automated feeding system data enable the derivation of biologically interpretable feeding behaviour and resilience phenotypes with measurable genomic signals. Within the present dataset, personality information did not provide additional predictive value beyond genomic relationships. Larger datasets and alternative modelling strategies, including models explicitly accounting for repeated lactations (e.g. repeatability or random regression models) or alternative personality indices, may help to further evaluate the potential role of personality in genomic prediction of feeding behaviour and resilience traits.

Genetic parameters of rumination and eating behaviour in Holstein cows monitored using collar-based precision livestock farming sensors

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Precision livestock farming (PLF) technologies enable continuous monitoring of behavioural traits that may serve as indicators of animal welfare, productivity and environmental adaptation. The aim of this study was to evaluate feeding behaviour traits recorded by collar-based sensors and to estimate their genetic parameters in dairy cattle. Behavioural data were collected using Aficollar (AfiAct II) sensors on three commercial Holstein farms located in Osijek–Baranja County, Croatia. After data editing, 26,462 daily behavioural records from 454 cows (n = 171, n = 147 and n = 136 across farms) were available for analysis. Recording periods differed among farms and ranged from May 2025 to January 2026. Mean rumination and eating times were 491 and 325 minutes per day, respectively. Environmental conditions varied substantially across farms, with temperature–humidity index (THI) values ranging from 37.9 to 84.4, representing winter conditions to summer heat stress. Correlation analysis showed a positive relationship between rumination and eating time, while both behavioural traits were negatively associated with THI, indicating reduced feeding behaviour under heat stress. Mixed model analysis further confirmed this pattern, with both rumination and eating time decreasing by approximately 2.2 minutes per unit increase in THI. Genetic parameters were estimated using pedigree-based repeatability animal models including fixed effects of farm, parity, days in milk and THI, and random effects of animal additive genetic and permanent environmental effects. Heritability estimates were low for rumination time ($h^2 = 0.04$) and moderate for eating time ($h^2 = 0.24$). Repeatability estimates were moderate for both traits (0.52–0.55), indicating consistent behavioural differences among cows. These preliminary results demonstrate that collar-derived behavioural traits capture meaningful biological variation and that eating behaviour shows moderate genetic variability, suggesting potential for inclusion of such traits in future breeding and management strategies. Ongoing genomic analyses will further support the investigation of genetic architecture underlying behavioural responses recorded through PLF technologies.

Big Data Meets Behavior: Genetic Characterization of AMS-Based Indicators in Dairy Cows

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Automated milking systems (AMS) continuously gain importance in dairy cattle herds which is mostly triggered by an increasing shortage of qualified labor. For AMS with free cow traffic, the behavior of cows to adapt to such systems is of high relevance and partly is determined by genetic effects. Apart from behavioral categories such as fear or aggressiveness, cows also need to learn that robot systems are programmed such that milking intervals cannot be too short as this would hamper the effectiveness of the AMS. AMS provide a number of parameters related to behavioral traits. Traits related to milking temperament will be udder scan time (UST) or connecting time (CT) while repeated short visiting intervals (VI) and a resulting high number of rejected milkings (RM) can be attributed to learning behavior. Aim of the present study was to define parameters from UST, CT, VI and RM and subsequently estimate genetic parameters as well as conduct a GWAS to unravel genomic regions of interest. Data was collected from 24 German dairy farms and covered a time span from 2007 – 2024. Data comprised 15,684 Holstein cows with 41,581 lactations and was aggregated on a daily basis to form 12,186,928 daily cow-AMS interactions. SNP genotypes were supplied by Vereinigte Informationssysteme Tierhaltung w.V. (VIT), Verden, and consisted of 10-K genotypes imputed to 50 K with 45,613 usable SNP. All cows were genotyped and phenotyped. For the estimation of genetic parameters under ssGBLUP, the BLUPF90 software was used. Heritability estimates were 0.1 (SE = 0.005) for RM and 0.25 (SE = 0.007) for VI, respectively, with a highly negative genetic correlation (-0.98) between them. Both traits are highly influenced by the ability of the cow to adapt to the time limits set for subsequent milkings and thus strongly determined by learning behavior. Furthermore, GWAS for the trait RM revealed a number of interesting regions on chromosomes 14 and 29 with several genes well known from model animal or human genomics. The traits UST and CT, which could be taken as indicators for AMS effectiveness exhibited lower heritabilities, ranging from 0.04 (SE =0.003) to 0.07 (SE =0.005), respectively. UST and CT had a positive genetic correlation of 0.76. GWAS for UST and CT revealed interesting regions on chromosome 8 and 26 with some genes related to fear behavior or stress.

Genetic parameters of feeder replacement traits from electronic feeders' records in cattle

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Feeder replacements, defined as the rapid succession of two different animals at the same feeder, can indicate competition for access to feed in group-housed cattle. However, replacement detection from electronic feeders depends on the time interval between consecutive visits: short windows (<20 – 30 s) are more likely to reflect competitive displacements, whereas longer windows increasingly mix competitive and non-competitive successions. We therefore aimed to derive feeder replacement traits from electronic feeder records in Nellore cattle, quantify the impact of replacement windows on trait distributions, estimate genetic parameters, and test social genetic effects. Electronic feeder data (Vytelle SENSE) were collected in feed efficiency trials from 1368 young male Nellore cattle (three selection lines; born from 2013 to 2024; 32 pens). To standardize exposure, records were restricted to the first 70 days on trial, and animals with <39 valid days were removed, resulting in 1363 animals and 7,462,183 cleaned feeder visits (85.5% of the original records). For each feeder within the pen, visits were ordered chronologically, and the next visit at the same feeder was identified; the second animal was defined as the follower. After discarding sequences with the same animal as follower or missing follower identification, 7,312,133 visits remained to compute 2 traits per animal across five-time windows (≤5, ≤10, ≤20, ≤30, ≤300 s): average daily replacement frequency (Rd5 – Rd300) and the logit-transformed proportion of visits as follower (LPR5 – LPR300). Outliers (mean ± 3.5 SD within contemporary group) were set to missing. Genetic parameters were estimated via ssGBLUP using a relationship matrix combining pedigree (5,068 animals) and genomics (3,860 animals; 383,343 autosomal SNPs). Fixed effects included contemporary group (birth year × pen) and line, with covariates for animal age at beginning of the trial and dam age at calving; total feeder visits (Rd traits) or valid days on trial (LPR traits) were additionally fitted. A social animal model was fitted for LPR traits to partition direct and social additive genetic effects. Replacement events followed the daily feeder-use pattern, peaking after fresh feed delivery. The mean follower proportion increased from 35.5% (≤ 5-s) to 94.9% (≤ 300-s), consistent with broader windows capturing near-continuous feeder usage. Heritability for Rd traits was low to moderate and window-dependent (Rd5 = 0.33 ± 0.05; Rd10 = 0.36 ± 0.05; Rd20 = 0.33 ± 0.05; Rd30 = 0.30 ± 0.05; Rd300 = 0.14 ± 0.05). LPR traits were consistently moderately heritable (LPR5 = 0.40 ± 0.05; LPR10 = 0.46 ± 0.05; LPR20 = 0.44 ± 0.05; LPR30 = 0.44 ± 0.05; LPR300 = 0.40 ± 0.05), with the highest estimate at ≤ 10-s. Genetic correlations were positive and strongest among adjacent windows (up to 0.98), but weakened when the 300-s window was involved (rg = 0.39 – 0.86). Social additive genetic variance for LPR traits was near zero (≤ 3×10□□) and did not improve model fit. Feeder replacement traits are heritable, scalable behavioral phenotypes in Nellore cattle, and the replacement window affects both trait meaning and genetic inference. In this dataset, 10-s window maximized heritability. These phenotypes can be extracted routinely on a scale from electronic feeders data. Study supported by FAPESP Process #2026/07564-7.

Relationship between daily milking records and health status of dairy cows

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Indicators derived from records generated by automated milking systems (AMS) are used for early indication of a health status of dairy cows. They can also be used to define new phenotypes for breeding a resilient and sustainable dairy population. Our aim was to evaluate several potential indicators based on AMS records and their relationship to the occurrence of common cattle diseases. The initial dataset contained more than 1.6 million records of 1,613 cows collected from March 2023 to December 2025 on three farms equipped with DeLaval AMS. Potential indicators included daily milk yield (DMY; mean = 33.2 kg per day), number of milkings (NM; 2.5 milkings per day), mean (ECmean; 4.6 mS/cm) and maximum (ECmax; 4.9 mS/cm) electrical conductivity (EC) of milk and natural log-transformed inter-quarter range (lnIQR = ln(ECmax/ECmin); 0.1). Pearson correlation coefficients showed a strong relationship between ECmean and ECmax (0.83), between DMY and NM (0.65) and between ECmax and lnIQR (0.63). The relationships between lnIQR and other traits were weak and negative (-0.15 with DMY, -0.11 with NM). The data were supplemented with records of mastitis (MAS; n = 682), reproductive disorders (REP; n = 286), hoof and leg disorders (HL; n = 374) and metabolic diseases (METAB; n = 149) occurrence. We used the GLM procedure (SAS 9.4; SAS Institute, Cary NC) to assess the impact of fixed effects on the variability indicators. The indicators differed significantly across farms (3 levels), years (3 levels; 1. 2023, 2. 2024 and 3. 2025) and seasons of milking (4 levels; 1. March–May, 2. June–August, 3. September–November, 4. December–February), parities of cows (3 levels; 1st, 2nd, 3rd and subsequent parities), breeds of cows (3 levels; 1. Czech Simmental, 2. Holstein, 3. crossbreeds) and lactation stages based on days in milk DIM (3 levels; 1. DIM0-100, 2. DIM101-200, 3. DIM201-305). We used the GLIMMIX procedure with a logit link (SAS 9.4) to evaluate the impact of selected indicators, fixed effects, and the random effect of cows on health status. The results showed a close relationship between health status and data obtained during robotic milking. Lower DMY was associated with an increased likelihood of disease occurrence, as expressed by estimated odds ratios (OR) and 95% Wald confidence limits (Cl) for MAS (OR = 0.92; Cl 0.91–0.92), REP (OR = 0.94; Cl 0.93–0.95), HL (OR = 0.96; Cl 0.95–0.97), and METAB (OR = 0.89; Cl 0.88–0.90). A decrease in milk production is one of the first signs of disturbed homeostasis, negative energy balance, or systemic inflammatory response. It may reflect both the direct impact of the disease on metabolism and feed intake, as well as changes in animal behaviour, such as reduced activity. An increased EC was associated with a higher probability of MAS with corresponding ECmean (OR = 4.31; Cl 3.88–4.79) and ECmax (OR = 2.8; Cl 2.70–2.95) values. LnIQR showed a very strong association with MAS, but the odds ratio was unstable (OR>999.99). LnIQR also showed associations with REP (OR = 11.73; Cl 2.72–50.65), HL (OR = 7.73; Cl 2.32–25.07) and METAB (OR = 22.06; Cl 4.15–117.37). Increased conductivity is a known consequence of inflammatory processes in the mammary gland, characterised by changes in epithelial permeability and increased ion penetration into milk. Uneven conductivity between quarters may reflect local pathological processes or subclinical inflammation, which can be a source of systemic stress and, secondarily, affect other physiological functions and health status of the animal. Supported by the Ministry of Agriculture of the Czech Republic, project QK24020269 and institutional support MZE-RO0723-V02.

ICAR/IDF Sensor Initiative: Guidelines and reference standards for using rumination sensor data in animal health and welfare - recommendations for sensor data use for genetic evaluation

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The use of on-farm sensor systems is generating an ever-increasing volume of detailed data. These data can support genetic evaluation as novel auxiliary traits and enable improvement of animal health and welfare, while strengthening transparency and sustainability in the dairy sector. The joint ICAR/IDF Sensor Initiative aims to develop harmonised guidelines to facilitate the effective use of sensor-derived data to improve animal health and welfare. Bringing together experts from the ICAR Functional Traits Working Group and the IDF Standing Committee on Animal Health and Welfare, the initiative represents key stakeholders from academia, breeding organisations, and the dairy industry. The main objectives of this effort are to develop and reach consensus on: standardised definitions, data editing, and terminology for health conditions and behavioural traits measured with sensor systems; data exchange standards that support interoperability along the dairy value chain and are aligned with recognised ICAR and IDF principles; best prediction guidelines for data collection, processing and analysis to support genetic evaluations, management practices and health and welfare assessments; and, validation protocols for evaluating sensor performance. This platform enables discussion of results, refinement of proposals and alignment with the needs of stakeholders. The initial outcomes of the rumination sensor use case include recommendations for integrating rumination data into genetic evaluations and breeding programmes, developing auxiliary and key indicator traits, and establishing reference standards for resilience, health, and welfare assessment in dairy cattle. Scientific background papers on the application of sensor data in genetics and breeding, as well as guidelines for data cleaning, have already been published. Close collaboration between stakeholder groups within the ICAR/IDF initiative supports the strategic goals of both organisations by promoting widely accepted, science-based guidelines that benefit the entire dairy value chain. This presentation will provide an overview of the detailed recommendations, focusing on those related to data and information requirements for the use of sensor data in genetics.

Three-dimensional imaging provides scalable body weight phenotypes for management and breeding in dairy cattle

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Precision livestock farming technologies enable large-scale phenotyping under commercial conditions. Body weight (BW) and BW change across lactation are biologically relevant indicators of maintenance requirement and energy balance but are rarely recorded frequently enough in commercial herds to support routine decision-making and genetic evaluation. Three-dimensional (3D) imaging systems allow BW to be estimated automatically, non-invasively, and repeatedly under commercial conditions. The objective of this study was to evaluate scalable BW phenotypes derived from the CowFIT 3D camera system (VikingGenetics, Randers, Denmark) for use in herd management and genetic evaluation in Nordic Holstein, Jersey, and Red Dairy Cattle. Body weight was predicted from dorsal 3D images collected automatically in commercial Danish dairy herds. A total of 11,047 complete lactations recorded between 2019 and 2025 in Nordic Holstein, Red Dairy Cattle, and Jersey cows were analysed. Across lactations, average BW ranged from approximately 436 kg in primiparous Jersey cows to 740 kg in multiparous Holstein cows. Individual BW trajectories were used to derive BW level and early- and late-lactation BW change. Genetic parameters were estimated using bivariate animal models. Predicted BW during the final week before slaughter showed strong phenotypic correlations with slaughterhouse-recorded live and carcass weights ($r \geq 0.89$), supporting the plausibility of routinely collected 3D-derived BW under commercial conditions. Body weight level traits were moderately to highly heritable (0.40 – 0.63; SE = 0.03 – 0.04), whereas BW change traits showed lower heritability (0.13 – 0.19; SE = 0.03 – 0.04) as expected. On average, primiparous cows lost approximately 23 kg BW, whereas multiparous cows lost up to 60 kg from calving to minimum BW. Cows predisposed to greater BW loss after calving also tended to regain more BW later in lactation. Body weight change traits showed negative genetic correlations with milk yield (r_g = -0.30 to -0.18), whereas phenotypic correlations followed the same pattern but were weaker in magnitude. Greater BW at primiparous calving was associated with higher milk yield, but this relationship diminished in later lactations. In contrast, average BW showed weak or slightly unfavourable relationships with milk yield, particularly at the genetic level in later lactations. These results demonstrate how scalable 3D-derived BW phenotypes can be used to characterize size and energy dynamics, with potential applications in genetic evaluation and herd-level management through improved monitoring of BW development and energy balance across lactation in commercial dairy systems. While BW level traits largely reflect maintenance requirements commonly accounted for in feed-efficiency evaluations, BW change traits may provide additional information on metabolic resilience.

Grazing traceability of dairy cows with GPS and accelerometer sensors.

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In France, dairy farms producing milk for specific quality schemes such as Protected Designation of Origin (PDO) cheeses and organic milk must comply with strict grazing specifications, including a minimum grazing duration (generally ≥170 days per year with ≥6 hours per day) and a minimum grazing area (≥30 ares per cow per year). Compliance is currently verified through periodic on-farm audits based on visual observations, paper grazing records and farmer declarations. These procedures are time-consuming and lack precision. Digital technologies, including Global Positioning System (GPS) and accelerometer sensors combined with advanced data analysis, offer new opportunities to automate and objectify grazing traceability. This study evaluated the potential of GPS and accelerometer sensors to assess grazing time and pasture use in five dairy farms located in the Maroilles PDO cheese area (France). During the grazing season, one or two cows per farm were equipped with two types of collars: (i) a GPS collar (RF Track) recording positions every 15 minutes, and (ii) a high-frequency GPS/accelerometer collar (Tauro, Embeint) recording positions every second with high spatial accuracy. The objectives were: (1) to quantify outdoor time, paddock use and effective grazing time; (2) to generate relevant indicators for grassland management; and (3) to evaluate the added value of high-frequency positioning and behavioural data compared with standard GPS monitoring. As a first step, high-frequency GPS data were used as a reference to classify cow location (barn vs pasture), complemented by farmer declarations. Density-based clustering methods were applied to 15-minute GPS data to automatically detect barn areas and estimate time spent outdoors. Across farms, mean daily outdoor time during the grazing season ranged from 7 to 20 hours, highlighting substantial variability in grazing practices within the same production area. Spatial overlap between GPS positions and paddock boundaries enabled the estimation of time spent in each paddock, allowing both the reconstruction of grazing calendars and the calculation of daily grazed surface per herd. As a second step, high-frequency GPS and accelerometer data were analysed to assess their added value. Behavioural classification based on movement and activity signals allowed discrimination between grazing and non-grazing activities while outdoors. This approach enabled estimation of effective grazing time, identification of under-utilised areas and refinement of paddock-level utilisation indicators. Compared to low-frequency GPS alone, the combined high-resolution positioning and behavioural data improved the accuracy and robustness of grazing traceability metrics. These results demonstrate that embedded sensor technologies, combined with adapted data processing methods, can provide reliable and automated tools for grazing traceability in quality-labelled dairy systems. Beyond compliance monitoring, such systems offer additional decision-support indicators for farmers and advisers to improve grassland management.

Effect of eating dynamics on enteric gas concentration recorded via sniffer in dairy cows

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Enteric CH4 emission is affected by the diurnal pattern of eating dynamics. The aim of this study was to assess the effect of a cow's visit to the main feed on the gas concentrations measured by a sniffer instrument. Lactating Danish Holstein cows were housed in a loose pen with mats and slatted floors. The cows had access to a partial mixed ration fed in automatic feeding bins (AFB) which recorded individual feed intake. In addition, cows had access to an automatic concentrate feeder (ACF), which was fitted with a CH4 sniffer with Guardian hardware (Edinburgh Instruments, Livingston, UK). Data from three months, December to February, of recording was available from the Danish Cattle Centre, AU Viborg, Denmark. Cow's visits had to meet these conditions: 1) had sniffer visits with at least two minutes of gas recording, 2) had visited both AFB and sniffer on the same day, 3) had a minimum of three meals per day from the AFB, and 4) had at least 14 days with valid data. A visit to a feeder was considered as a meal if there was a minimum of 30 min break between the two consecutive visits to the same type of feeder. The sniffer-recorded gas concentrations were processed using the GEDA software to ensure a high quality data. Finally, AFB and ACF data were merged in chronological order, and time distance (TD) as min between a sniffer visit and the last meal at the AFB were calculated. In some instances, a visit to the ACF happened during a meal at the AFB, in which case the TD was considered 0. If a cows visited the AFB before going to the ACF, the feed intake as kg fresh matter, approximately 40% DM, from the AFB was associated with the visit to the sniffer, and defined as last feed intake before gas measurement (LF). The data was analysed with a generalized mixed linear model with CH4 (ppm), CO2 (ppm) or CH4:CO2 ratio as dependent variable using a Gamma distribution with log link. The explanatory variable of the model included a mean intercept, the regression coefficients for centred TD, centred LF, the interaction between the two, and the squared LF, as fixed effects. The random effects included the regression coefficients for centred TD and LF within cow, and the random intercepts for cow and date. The CV for the residuals was calculated as a percentage of the ratio between the SD of the residuals and the average of the observed variable. Data from 34 cows was retained, with an average of 32.8 ± 11.8 day per cow (14 – 61 d, min – max). The concentration of CH4 was affected by TD and LF ($p = 0.04$ and $p = 0.01$). Increasing TD and LF increased CH4 concentration with 0.29 10^{-3} ppm/min for TF, and 0.68 10^{-3} ppm/kg for LF. Similarly, the concentration of CO2 was affected by TD and LF ($p < 0.001$ and $p = 0.01$). Both increasing CH4 concentration with 0.50 10^{-3} ppm/min for TF, and with 6.1 10^{-3} ppm/kg for LF. The CH4:CO2 ratio decreased with increasing TD ($p < 0.003$, −0.24 10^{-3} ppm/min). The effect of LF was not significant, but it tended to increase the CH4:CO2 ratio ($p = 0.07$). The CV for the residuals on the mean were 51, 38, 32% for CH4, CO2, and CH4:CO2. This study indicates that eating dynamics affects gases concentrations measured from sniffer. However, the eating dynamics and random effect of date and individual cow left up to 50% of the gas concentration unexplained. Using the CH4:CO2 reduced the residuals error.

Non-invasive infrared measurement of exhaled methane in dairy cows based on spot sampling during automatic milking

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Ruminants are essential to sustainable agriculture by converting human-indigestible plant biomass into high-quality animal protein, yet their enteric methane emissions substantially contribute to the sector's climate impact. Robust and practical measurement technologies are needed to support mitigation strategies under commercial conditions. This study evaluated the effectiveness of a non-invasive methane sensing approach based on spot sampling, which was integrated into an automatic milking system for German Holstein, Fleckvieh, and crossbred dairy cows. Between April and December 2025, breath samples were continuously drawn via a small sampling probe during milking and analysed in real time using infrared detection (IRD). Methane prediction values obtained from routine milk recording provided by the Bavarian State Association for Animal Production (Landeskuratorium der Erzeugerringe für tierische Veredelung in Bayern e.V.; LKV) were used as the reference standard. A total of 425 observations from 148 cows recorded on six performance test dates were analysed. Prior to regression analysis, a mixed model analysis was performed using SAS 9.4, including the random effects cow and test date, as well as the fixed effects genetic group and the covariables days in milk, energy corrected milk, IRD minimum (IRD-Min) and IRD maximum (IRD-Max). In addition, stepwise regression analysis using the same variables (excluding the fixed and random effects) confirmed that all the predictors were significant contributors to the model, yielding an R2=0.56 [root mean square error (RMSE) 39.6 l/d]. After applying a breed-specific correction factor, the coefficient of determination improved to R2=0.59 (RMSE = 30.3 l/d). LKV-derived methane estimates showed a relatively high association with the predictors derived from the mixed model analysis containing the infrared-based methane measurements (IRD-Min, IRD-Max) as co-variables (R2=0.64, RMSE 35.4 l/d) – demonstrating substantial predictive capacity despite on-farm variability. These findings provide compelling evidence that infrared-based, non-invasive breath analysis during milking could become a robust and scalable method for quantifying enteric methane emissions in dairy cows and holds promise for integration into large-scale monitoring and mitigation frameworks.

Broader potential applications of sniffer systems: A pilot study of installation on automatic feeder station

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The implementation of sniffer systems for monitoring enteric emissions is supporting genetic improvement programmes for the selection of low-emitting animals within the context of global greenhouse gas mitigation strategies. Over recent years, trials involving sniffers have commonly been associated with the presence of automatic milking systems (AMS), facilitating the collection of emissions data in dairy production systems; however, limiting their application to farms with AMS. Automatic feeder stations (AFS) equipped with individual animal identification systems and structural containment can also be suitable for sniffer installation, as they recognize and maintain the animal isolated for defined periods during the day. Nevertheless, animals' use of AFS differs from the use of AMS in terms of visit duration and daily frequency; for this reason, appropriate sniffer data management strategies need to consider these conditions. This research aims to provide a pilot trial of enteric emission monitoring using a sniffer installed on an AFS and to compare the monitored emission levels to those estimated using the IPCC Tier 2 equations. The trial was conducted on a dairy farm rearing Holstein-Friesian and Pezzata Rossa cows, where a sniffer (MooLogger, Tecnosens IT) was installed on an AFS (DairyFeed C8000, GEA DE). Data on emissions, individual production and physiological state were gathered from 70 lactating animals between July 2025 and January 2026. The mean duration of animal visits to the AFS was 00:01:25, with an average frequency of 5.5±3.5 visits/head/day. Visits lasting less than one minute were discarded, as they were not consistent for evaluating CH4 and CO2 patterns. Sniffer data were aligned with AFS recordings using timestamps, and gas measurements were processed by removing outliers, negative values, and background gases. To detect eructation events, CH4 peaks were identified, showing an average of 1.43 ±0.70 peaks per visit. Visits with at least one peak were selected and CH4 emissions as grams per day were calculated through the CH4-to-CO2 ratio. Preliminary results indicate an 11% discrepancy between emission levels detected using the sniffer and those estimated with the IPCC Tier 2 equation. Mean CH4 emissions calculated from sniffer data were 349.8 ± 177.6 g/head/day, referring to a CH4-to-CO2 ratio of 0.09±0.04, whereas emissions estimated using the Tier 2 equation averaged 387.1 g CH4/day. Further research is needed to confirm these results, and different approaches can be explored to improve the quantification of CH4 emissions from AFS visits using sniffer data. The use of sniffer systems on AFS could represent a promising step toward enhancing in-vivo monitoring of enteric emissions in ruminant production systems, broadening the range of dairy farms that can participate in monitoring programmes and extending this capability to beef systems.

Proxies for early lactation ketosis in Austrian Fleckvieh dairy cattle based on daily milk yield and rumination data

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As the use of automatic milking systems (AMS) and sensor systems for herd management increases, the potential use of data from these systems for breeding purposes is being investigated more and more. There is particular interest in the potential to derive phenotypes that can be used as proxies for traits with low heritability and/or costly phenotyping. Due to the low frequency of diseases, functional traits related to dairy cow health are the perfect example of traits with low heritability and challenging phenotyping. In this study, we investigated the potential of using traits derived from daily milk yields and daily rumination time data to predict early lactation ketosis in Austrian Fleckvieh dairy cows. AMS data were available for 5,436 cows from 66 commercial dairy farms, 25 (1,727 cows) of which were also equipped with Lely sensor systems. Results from blood tests measuring β-hydroxybutyrate (BHB) levels were used as a reference for ketosis (available for 68 farms and 3,359 cows). In addition to BHB concentrations in mmol/L, a binary coding approach was applied to the ketosis level trait: concentrations exceeding 1.2 mmol/L indicated a state of (subclinical) ketosis (1), while concentrations below this threshold indicated a healthy state (0). As rumination curves resemble lactation curves, we investigated the potential of Wood function parameters and individual deviations from it to define auxiliary traits for early lactation ketosis using milk yield (MY) and rumination time (RT) data. First, we estimated the single parameter b, representing the ascending slope in the Wood function, for MY and RT for each cow within the first 30 days of milk production and calculated the ratio between these two. Additionally, Wood curves were fitted for all animals in parity 1 and for animals in higher parities, respectively, in a mixed model approach including animal (and lactation for animals in higher parities) as random effect and a correcting for autocorrelation of repeated measurements within the same lactation. The traits based on the residuals of each animal were defined as follows: the sum of the daily residuals divided by the number of days, and the ratio between the rumination and milk residual traits. Heritabilities were 0.18 (±0.03) for BHB blood levels and 0.12 (±0.03) for the binary ketosis trait, 0.09 (±0.02) for bMY and not significantly different from 0 for bRT. Due to the high genetic correlation between the two traits (-0.78 ±0.28), estimates for the ratio between them were not considered reliable. A negative genetic correlation was found between bMY and blood BHB level (-0.62 ±0.17). Based on this high genetic correlation and the presence of AMS on many dairy farms this trait may be considered as potential auxiliary trait for ketosis. Further results and genetic correlations with other traits in the total merit index will evaluate the suitability for routine genetic evaluation for metabolic stability in dairy cows.

AI-based 3D monitoring of behavior and welfare indicators in cows during the preclinical phase of atypical BSE

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Atypical bovine spongiform encephalopathy (BSE), also known as “mad cow disease”, continues to challenge animal health, food safety, and fundamental research. In an ongoing experiment investigating the pathogenesis of atypical BSE, 20 young cows were infected with either H- or L-type atypical BSE by intracerebral inoculation. The clinical onset of the disease, characterized by typical neurological symptoms, is expected after an incubation period of approximately 12 to 20 months. However, subtle alterations may already occur during the preceding preclinical phase. These include behavioral changes such as apathy, social withdrawal, or compulsive patterns, as well as early physical warning signs including mild locomotor impairment, loss of condition, and increased sensitivity. The objective of this project is to investigate the preclinical phase of atypical BSE in experimentally infected cows and to identify subtle deviations in behavior and species-specific locomotion prior to the onset of overt clinical signs. Particular emphasis is placed on integrating animal welfare indicators into the analysis, including activity levels, social interactions, spatial use, posture, and movement quality. The study aims to detect quantifiable behavioral and movement parameters that may serve both as digital biomarkers of emerging neurological dysfunction and as objective indicators of animal welfare status. To this end, a computer-assisted monitoring framework based on state-of-the-art computer vision methods is being developed. The central goal is to establish and validate a robust, data-driven model for automated longitudinal anomaly detection, enabling the identification of progressive deviations from individual and group-specific baseline patterns under practical housing conditions. The cows were housed in an experimental barn similar to a conventional dairy cubicle system, without a milking unit. Continuous recording was achieved using four synchronized cameras installed at the barn corners. Multi-camera calibration enabled 3D reconstruction of relevant body points for quantitative analysis of gait, head posture, activity, spatial behavior, and social interactions. YOLO-based convolutional neural networks were trained to detect and track animals and to extract movement-related features. The system allowed reliable individual tracking under practical conditions and generated sufficiently accurate 3D movement data. Initial anomaly detection models identified significant deviations from individual baselines preceding clinically evident neurological symptoms. AI-supported 3D behavioral analysis demonstrates considerable potential for the objective detection of subtle preclinical changes in atypical BSE. Continuously collected movement, behavioral, and welfare-related parameters may serve as digital biomarkers for early neurological dysfunction while simultaneously providing an objective framework for continuous animal welfare assessment. Further research is required to validate model specificity and sensitivity and to distinguish disease-specific effects from age-, developmental-, or environment-related influences. This approach integrates experimental prion research with AI-supported behavioral analytics and offers novel perspectives not only for the automated early detection of neurological diseases in livestock but also for data-driven, continuous animal welfare monitoring under practical farming conditions.

Sniffing the sustainable dairy future: experiences establishing methane emissions monitoring in Swedish dairy herds

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Issues with mitigation of methane from ruminants are ongoing worldwide. Since 2025 a Nordic Methane index based on Danish data is available in the Nordic Cattle genetic evaluation. While feed additives have a transitive effect and may cause digestive issues for some cows, breeding for an optimized balance of health, milk yield and methane production can produce effects that are permanent and sustainable. During 2025, the Swedish University of Agricultural Sciences (SLU) collaborated with Växa and Aarhus University (AU) within the Global Methane Genetics initiative to install gas sniffers (Guardian NG, Edinburg sensors) on five Swedish dairy farms attached to the SLU cattle data infrastructure Gigacow. This represents the first effort to establish large-scale dairy methane emissions monitoring in Sweden with the purpose of defining breeding values for methane production, with a focus on the local Swedish red and white dairy breed. Integrating the UK-made instrumentation and Danish data collection pipeline with two Swedish cattle databases and five active commercial dairy farms presented a variety of technical and organizational challenges. Here we present our strategies and experiences, and implications for further expansion of methane monitoring as well as international sharing of data. There are a small number of brands of automated milking systems (AMS) in use in Swedish dairy herds, but a greater variety of floorplans in dairy barns. This creates challenges in positioning gas sniffer units and connecting them to their corresponding AMS, power supply and wireless internet. In some cases, ad hoc modifications were necessary to fix sniffers atop AMS units. Sniffers manufactured for the Danish market also had to be modified in the field to add Swedish standard power plugs. The sniffer units were configured to transmit daily batches of output to SLU Gigacow, which was expanded to integrate gas data into the existing SQL database. Several issues with robot, sniffer and farm identities had to be accounted for when constructing the gas data import functionality. An application programming interface was developed along with an online dashboard (available at https://gigacow.slu.se/ – cancel the login prompt to access public data) to provide summary statistics and enable remote monitoring of sniffer function. Work is ongoing to combine cow identities recorded by AMS with the continuous gas measurements from the attached sniffer unit, using a pipeline developed at AU. The lessons learned from installing sniffers at a single pilot farm in south-central Sweden accelerated the installation procedure at a subsequent four farms around the southern half of the country. The remote monitoring system developed by SLU will allow a small team of technicians at Växa to maintain sniffers as needed and relocate them to new farms. Thus Swedish researchers and animal breeders can make a case for regular methane monitoring at dairy farms, define methane efficiency phenotypes in local and international breeds, and improve the sustainability of future dairy herds.

Combining population and quantitative genetics to improve genomic prediction

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In breeding populations, the temporal dynamics of a causal allele for a selected trait results from a complex interplay between its effect on the trait, its frequency, the effects and frequency of other causal alleles and the evolutionary constraints affecting individuals (selection, drift, pedigree structure). These parameters will, in turn, also affect the estimation of marker effects and breeding values. However, they are typically not fully accounted for in genomic prediction (GP) models. In this work, we propose to improve genomic prediction models by incorporating additional information on the selection coefficients of the markers, estimated using a method from population genomics. This method, called selnetime (Uhl, Bunel et al. 2025), uses the temporal dynamics of the allele frequency trajectories through generations of selection to estimate a selection coefficient measuring the relative fitness of individuals carrying a particular allele. In a breeding population, we expect the favorable alleles for the trait to increase in frequency over time and therefore have positive selection coefficients. We developed a new statistical approach to re-estimate marker effects from modeling their joint distribution with their selection coefficient, using a meta-analysis method (mash, Urbut et al. 2019). We evaluated the performance of this new approach on simulations of a diverging selection experiment inspired by a real dataset. The selected trait is controlled by both a QTL and a polygenic effect. Additional neutral markers are also simulated, to evaluate the effect of selection on allele frequency dynamics solely due to the pedigree structure resulting from selection across generations. We show that state-of-the art statistical methods for GWAS/GP lead to non-null correlation between quantitative and adaptive effects, not only for the QTL but also for the neutral markers. This latter correlation results from population structure and leads to an overestimation of the estimated breeding values. Re-estimating the quantitative effects with our new approach removes most of the correlation for the neutral markers while preserving good performance at estimating true QTL effects. Overall, our proposed model improves genomic prediction by inducing an additional shrinkage on non-causal variants while preserving true QTL effects. We will discuss the implication of our results for managing breeding populations, in particular its relevance for the classically observed erosion of GEBVs or for cross-population genomic prediction.

Breeding Perspectives on Red and White Dual-Purpose Cattle: Results from a Multinational Survey

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Many local cattle breeds are endangered and some under the thread of extinction. The strong focus on milk production has led to a shift away from local dual-purpose breeds towards highly specialised dairy breeds. However, there are important arguments for the preservation of these local breeds such as maintaining genetic diversity and the adaptability in the face of changing environmental conditions. Red and White Dual-Purpose cattle (RWDP) was formerly spread across the Northwestern part of Germany (GER) but declined strongly in this region. At now, the largest RWDP population in GER is registered in Schleswig-Holstein (SH). Due to the relatively small population size, cooperation with closely related breeds is necessary to establish a reasonable reference population for genomic selection to be competitive. Due to their close relationship Rouge Pie de l´Est from Belgium (BEL) and Pie Rouge de l´Oesling from Luxembourg (LUX) were used in this study (hereinafter collectively referred to as RWDP). Little is known about population differences as well as the breeding perspectives for these three breeds. A multinational survey covering farm and sociodemographic characteristics, herd size and structure, management and breeding aspects and future opportunities was conducted among breeders in GER, BEL and LUX. The questionnaire was accessible from March to May 2024 and reopened for BEL and LUX from October 2025 to February 2026 as no initial responses were received. Overall, 28 (GER), 6 (BEL) and 1 (LUX) questionnaires were answered. In total, the participants kept 1,724 (GER), 290 (BEL) and 60 (LUX) RWDP cows. In GER, 82.5% of the answers came from SH, while in BEL all responses originated from the province of Liége. The proportion of GER farms keeping only purebred RWDP cattle was rather low (22.2%) compared to BEL (100%). In GER, the additional breed in mixed herds was mostly Holstein Friesian. The average herd size was 95 (range: 20-215) in GER, including on average 64 RWDP cows (range: 1-200), 73 (range: 60-110) in BEL and 63 in LUX, including 60 RWDP cows. The RWDP average milk yield is 7,000 kg (range: 5,000-8,000 kg) for GER, 6,083 kg (range: 5,000-7,000 kg) for BEL and 6,184 kg for LUX. Breeding progress (GER) and inbreeding (BEL) were identified as the main future challenges. GER and LUX breeders prefer to maintain type, whereas most BEL breeders aim for a stronger dairy type. Improvement of udder traits was the main priority in GER, while BEL and LUX focused more on output traits such as milk volume, milk components and meat production. Across regions, breeders agreed on the need for a sufficient number of elite sires. Current selection of dams emphasises milk yield and longevity (GER and BEL) and dual-purpose character (LUX). Most breeders would prefer genomic breeding values for selection decisions. Despite the current trend some aspects raise hope for RWDP in the near future. Most breeders are planning to maintain herd sizes and envisage a slight increase in RWDP proportion within herds. Additionally, 79.7% already have a potential farm successor, particularly among farmers aged >55 years. However, the strong confinement to specific regions within country poses a significant long-term risk.

Impact of relationship matrices on inbreeding control in pig populations under optimum contribution selection

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Maintaining genetic diversity is a major challenge in animal breeding programs. Optimum Contribution Selection (OCS) is among the most established strategies for balancing genetic gain with the preservation of genetic diversity by limiting the buildup of inbreeding across generations. However, the effectiveness of OCS depends directly on how relationships among selection candidates are defined. In general, the methods to construct the relationship matrix can be classified into 3 approaches: (i) IBD-based methods estimate the probability that alleles are identical due to common ancestry, (ii) Drift-based methods quantify deviations in allele frequency from a base population due to genetic drift, and (iii) Homozygosity-based (HOM) methods estimate genome-wide homozygosity. The choice of relationship matrix determines how the different inbreeding approaches (IBD, Drift, or Homozygosity) will be constrained. Theoretically, FDRIFT and FHOM estimated at neutral loci should be equal when there is no selection. Deviations between these estimates can indicate the impact of selection on genetic diversity, providing insight into whether breeding strategies effectively maintain genetic variation over time. The aim of this study is to evaluate the performance of different relationship matrices in OCS by comparing their effects on genetic gain and inbreeding. The modular breeding program simulator (MoBPS) will be employed to simulate breeding populations. Genomic selection will be based on breeding values estimated using a genomic relationship matrix (G), while six alternative relationship matrices will be used exclusively within the OCS framework to manage genetic diversity. The simulated population will reflect a pig breeding population, including a nucleus population of approximately 120 animals per generation, simulated over 10 discrete generations under genomic selection with OCS to control inbreeding. The genome will be distributed across 18 chromosomes. A total of 5,000 SNP loci will be sampled as marker loci for genomic prediction, 1,000 as additive QTL with allelic effects sampled from a normal distribution, and 1,000 as neutral loci to assess allele-frequency changes and loss of heterozygosity. Six matrices will be used for OCS, namely 2 IBD-based matrices (pedigree relationship matrix (A), and genomic relationship matrix based on linkage analysis (GLA)), 2 drift-based matrices (genomic relationship matrices (G) based on VanRaden methods 1 and 2), and 2 homozygosity-based matrices (runs of homozygosity matrix (GROH), and G matrix with allele frequencies fixed to 0.5 (G0.5)). Obtained genetic progress and inbreeding rates will be investigated using both drift-based and homozygosity-based approaches at neutral loci. The results of this study are expected to identify the most suitable relationship matrix for OCS and to provide practical recommendations for pig breeding programs to sustain genetic progress while controlling inbreeding rate, thereby ensuring the long-term sustainability of genetic improvement.

Performance of optimal contribution selection depends on the accuracy of selection candidates

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Optimum-contribution selection (OCS) balances genetic gain and inbreeding, but its performance depends on the accuracy of genomic evaluations for selection candidates. We quantified how accuracy changes the gain-inbreeding frontier under OCS in a simulated dairy breeding program with an open breeding nucleus. The breeding goal included milk production (h^2 = 0.3) and a functional trait (h^2 = 0.04) with a negative genetic correlation (rg =-0.30). The traits were weighted equally in the breeding goal (~83 per additive genetic standard deviation). A base population of unrelated animals was simulated and assigned to age classes 1-5 years for females and 1-6 years for males. The nucleus consisted of 10,000 lactating cows and approximately 4,500 replacement heifers across 100 herds. Each year 500 one year old males and 2,000 one year old females were genotyped based on parental average of genomic estimated breeding values (GEBV). Bull calves produced via MOET were given priority for selection. Active sires for mating were selected using OCS from the 500 genotyped males. The number of selected sires was determined by the OCS algorithm. Among the genotyped females, 100 with the highest GEBV were chosen as MOET donors, each mated to two sires to produce a total of ten offspring. To vary evaluation accuracy while keeping genetic parameters, population structure, and the breeding scheme fixed, we used a pseudo-genomic evaluation: candidates' GEBV were constructed as noisy predictors of true breeding values (TBV) so that cor(GEBV, TBV) equaled 0.99 (High), 0.70 (Medium), or 0.50 (Low), rather than being obtained from an explicit SNP-based prediction model. The breeding schemes were simulated for 15 years. OCS maximized with g_T C+λ 1/2 c□ Ac with λ {0,-1,-3,-5,-10,-25,-50,-70,-85,-100,-1000}. Outcomes were summarized across replicates and years as 1) annual genetic gain (ΔG), estimated as the slope from a linear regression of TBV on year and 2) the rate of inbreeding (ΔF), estimated from the slope s of the linear regression of ln(1–F) on generation ($\Delta F=1-e_s$). Results showed that the shape of the frontier varies across accuracies. The marginal increase in ΔG by increasing λ from -1000 to -50, were 12.7; 8.3; 6.8 for High, Medium and Low. For penalties lower than -50, increase in ΔG was low. Overall, ΔF varied from 0.14% and up to 0.71% for High, 0.14% to 1.00% for Medium and from 0.16% up to 1.20% for Low per generation across all penalty levels, demonstrating that the maximum achievable ΔF along the frontier decreases substantially with higher accuracy. The preliminary results indicate that the performance of OCS is improved remarkably with higher selection accuracy of selection candidates. In practical breeding schemes, where selection candidates vary in evaluation accuracy across cohorts, further investigation is needed.

Surveying and evaluating UK farm animal genetic diversity estimates

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Local and rare livestock breeds are highly adapted to niche environments and contribute more than expected to overall species diversity. These breeds are reservoirs for the genetic variation needed to face future challenges. Monitoring the genetic diversity found in these populations is key for prioritising resources for conservation purposes. In the UK, there are three sources of information for monitoring: annual diversity estimates published by the Department for Environment, Food and Rural Affairs (DEFRA); an annual watchlist published by the Rare Breeds Survival Trust (RBST), a charity that conserves and promotes native UK livestock and equine breeds; and diversity estimates published in peer-reviewed scientific literature. Our aims were to: (1) summarise genetic characterisation (effective population size (Ne) and inbreeding) of domestic populations in the UK and Ireland; (2) identify populations on the RBST watch list that have genetic diversity estimates in peer-reviewed literature; and (3) compare DEFRA's annually reported (census-based) estimates of Ne with (inbreeding- and DNA-based) estimates from peer-reviewed literature. We identified a total of 37 studies published from 1975 to 2024 with genetic diversity estimates. While 42% of the breeds on the RBST watchlist have been included in peer-reviewed publications with genetic diversity estimates at least once, there were still many breeds, and even a few species (goat, turkey, duck and geese) with no available pedigree- or DNA-based genetic diversity measures. In contrast, 79% of breeds have at least one census-based Ne estimate published by DEFRA. We found census-based Ne estimates were, on average, higher than DNA-based estimates, likely due to violated assumptions in the census-based methodology, because of the structure of livestock mating systems. While breeds identified by the RBST as the highest priority for conservation had similar Ne census-based and DNA-based estimates (average difference 7), the difference increased as the priority level of the population decreased, with the census-based estimates eight times higher than the DNA-based estimates for the lowest priority level (average difference 1468). We recommend that, where possible, DEFRA switch to F-based estimates, and eventually to DNA-based estimates as resources become available. Most of the peer-reviewed publications estimated genetic diversity measures using pedigree, microsatellite markers, or SNPs. However, to identify breed-unique variants responsible for adaptive traits in rare breeds, more studies using whole-genome sequencing will be needed. Having genetic diversity estimates are an important step to identifying breeds at risk, however, future resources should also be directed toward implementing breeding programmes with the aim to conserve genetic variation and unique traits, and improve functional traits. This will require not only pedigree information (and genomic information if possible), but phenotypic records as well.

A genomic survey of the Romagnola beef cattle breed

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Romagnola is an indigenous Italian beef cattle breed, reared in the Emilia-Romagna region. Although it was considered one of the most promising European beef breeds between 19th and 20th century, it progressively faced a reduction in its use and population. As of 2024, only 8,691 animals are registered in the herdbook. Due to the reduced population size, the risk of genetic erosion is considerable. This work aimed to explore the genomic conservation status of Romagnola breed; to this end, the complete set of genotyped Romagnola animals, consisting of 2,644 animals, and a common panel of 25,445 SNPs was used. The genetically close Marchigiana and Chianina breeds were used for comparison in population structure (i.e., PCA, linkage disequilibrium decay, pairwise FST values, admixture, computed via PLINK v1.9 and ADMIXTURE software); observed and expected heterozygosity, alongside fixation indexes FIS were also obtained via PLINK software, detectRUNS R package was used to detect runs of homozygosity, while SNeP1.11 software was used to estimate the effective population size. Results indicated, beside an evident genetic similarity with the other two breeds, a strong genetic identity for Romagnola, being the first to be differentiated by ADMIXTURE, and no faster linkage disequilibrium decay compared to the Chianina breed, which has not undergone genetic bottlenecks as Romagnola. FIS values resulted in an average of -0.005, confirming the lack of inbreeding and no relevant deviation from Hardy-Weinberg equilibrium. Nonetheless, the trend for such value across the years shows a progressive increase, although FIS is still close to 0, reaching 0.009 in 2024. Regarding the runs of homozygosity, on average each animal showed 27 regions under selection, with an average of 8.3 Mb of length, with the higher percentage of longer runs indicating more recent selection and inbreeding. Finally, the effective population size was 156 as of 13 generation ago, and a constant and linear decrease in the past 50 generations can be noticed. The analysis of genetic and genomic diversity in Romagnola breed did not evidence severe inbreeding conditions, but its increasing trend suggests progressive genetic erosion. Moreover, since its reduced population, and the constant and progressive decrease in census, the continuous monitoring is fundamental to support the efforts to preserve and valorize this breed. Funding: Regione Emilia-Romagna. Deliberazione della Giunta regionale n. 553/2025. Programma Operativo Regionale Triennale. Determinazione n° 23607 del 28 novembre. CUP E98H25001250009.

Seven decades in liquid nitrogen: Viability and fertility of historic bovine semen

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Gene banking of farm animal gametes serves as a critical safeguard for biodiversity, providing a biological insurance policy against diseases, climate change, and the loss of heritage lineages. While cryopreservation of semen is an established, routine procedure for long-term storage of male genetics, the theoretical time limits of storage are rarely tested over spans exceeding several decades. Validating the viability of long-term stored gamete samples is essential for confirming the efficacy of gene banks. This report details the functional analysis of semen samples of three German Black Pied cattle (DSN) bulls born in 1952, 1953, and 1966. These samples remained in cryostorage for 60 to 70 years. To evaluate the impact of ultra-long-term storage, the historic samples were compared against a control bull whose semen was cryopreserved in 2011 under modern EU standards. For each sample, semen quality parameters were assessed post-thaw. Kinetic data were collected via computer-assisted sperm analysis (CASA). Structural integrity was evaluated through flow cytometry, simultaneously assessing viability and acrosome integrity. To investigate potential cumulative genetic decay, DNA fragmentation was quantified using the sperm chromatin structure assay (SCSA). Furthermore, the functional competence of the historic germplasm was tested through in vitro embryo production (IVP) using oocytes retrieved from slaughterhouse ovaries. Post-thaw analysis revealed that while kinetic parameters presented a chronological decline, structural and genetic integrity remained remarkably high after ultra-long-term storage. Total motility for the bulls born in 1952 and 1953 was 17.1% and 14.0%, respectively, compared to 46.9% for the 1966 bull and 56.5% for the control bull. Flow cytometry confirmed that 16.5% (1952), 7.1% (1953), and 22.9% (1966) of the sperm populations maintained intact plasma and acrosome membranes. Most notably, the DNA fragmentation index (DFI) remained exceptionally low across all historical samples, comparable to the control sperm (0.5-1.0% vs. 0.1%). Morphologically normal spermatozoa were present at rates of 65.0% to 76.5% in the historical samples. Functional competence was confirmed through IVP, where the historic samples successfully achieved fertilization and progression to the blastocyst stage. The 1952 and 1953 samples each produced 10 blastocysts, while the 1966 sample produced 9 blastocysts. All embryos reached the blastocyst stage by day 7/8 and were classified as quality 1 or 2, suitable for cryopreservation. These findings validate the long-term efficacy of gene banking and confirm that germplasm from the pioneering era of cryopreservation remains a high-quality, biologically viable resource for modern biodiversity restoration and the genetic conservation of heritage breeds such as the German Black Pied cattle.

Genomic diversity and population structure in Nordic Red Dairy Cattle populations

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The Nordic Red Dairy Cattle comprises two main populations: the Red Dairy Cattle (RDC) and Norwegian Red (NR). The RDC breeding program, coordinated by VikingGenetics, unites Danish Red (DNK), Finnish Ayrshire (FIN), and Swedish Red (SWE) cattle within a joint genomic selection program. The NR breed (NOR), managed by Geno SA in Norway, has been developed independently under similar breeding objectives and with comparable selection intensity. Both breeding programs are supported by long-standing national recording and evaluation systems, which have enabled early adoption of genomic selection and a shared emphasis on production efficiency, health, and functional traits. Intensive selection within relatively small breeding populations, together with historical gene flow between the RDC and NR populations, has likely influenced patterns of genetic diversity and relationships among individuals, yet the extent and nature of these changes have not been comprehensively assessed. This study investigates the population structure and genomic diversity of RDC and NR AI bulls using medium-density SNP genotype data. The bulls were categorized into three groups: Group 1 represents animals born before the combined Nordic breeding program for RDC (prior 2009); Group 2 includes bulls from the era of the joint RDC program but before the implementation of genomic selection; and Group 3 consists of bulls after the full-scale adoption of genomic selection from 2015 onwards until the end of 2025. Population structure was characterized using Principal Component Analysis (PCA) and ADMIXTURE, while genetic differentiation was quantified using Fixation Index (FST). The PCA results revealed a clear trend of population unification within the RDC over time. Group 1 showed distinct clusters for DNK, FIN, and NOR bulls, with SWE bulls positioned between the FIN and NOR. In Group 2, the DNK and FIN bulls began to cluster more closely with the SWE bulls. By Group 3, the RDC bulls displayed substantial genomic convergence, reflecting the long-term impact of coordinated selection within the unified Nordic program. The NOR bulls consistently formed a distinct clade, maintaining genetic separation from the RDC across all three eras. ADMIXTURE analysis, conducted up to K=14, showed no clear plateau in cross-validation error, indicating a complex, highly admixed history of these populations. Despite this, clear group-specific trends were evident: Group 1 bulls displayed distinct country-specific admixture profiles, while in Group 3, the RDC bulls exhibited highly similar genomic compositions across countries. Throughout these eras, the NOR maintained a distinct admixture profile. These structural shifts were corroborated by FST analysis, which identified FIN and SWE populations as the most closely related. The highest historical differentiation was observed between DNK and FIN bulls in Group 1 (FST = 0.062), showing the genetic distance before integration. Furthermore, significant genetic drift within countries was evident over time, as seen in the FST distance of 0.034 between DNK bulls in Group 1 and Group 3. These findings show that past and ongoing breeding strategies have driven increased genomic similarity within the RDC population, while the NR population has maintained its distinct genetic profile. Together, the results reflect the long-term effects of selection within each breeding program and highlight the importance of continued monitoring of genetic diversity in both populations.

Global Variability of the Chicken D-Gene Region

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New emerging diseases and the increasing societal demand to keep chickens in less protected environments fosters the need for chickens with an immune system that allows reaction to those challenging environmental impacts. One critical component for an adaptive immune system is a variable repertoire of naive B-cells, which allows the generation of a broad spectrum of antibodies. Thereby, the antibodies consist of a heavy and a light chain. The light chain is encoded in the genome by a VL and a JL segment, while the heavy chain consists of a VH, DH and a JH segment. In vertebrates, immunoglobulin (Ig) diversity originates from the somatic recombination of the V- D- and J-gene segments in the Ig heavy chain locus, and V- and J-gene segments in the light chain locus with antibody diversity, in mammals further expanded by gene conversion. In contrast to mammals, chicken possess only one functional VH gene, followed by a cluster of DH segments and a single functional JH segment, limiting the combinatorial potential of V(D)J recombination. To date, the only published study on the native reservoir of chicken D-genes by Reynaud and Weill (1991) describes 16 distinct D-genes (very homologous D1-D15 and the slightly more different Dx) identified through Sanger Sequencing of chicken B-cells. However, only nine copies of D-genes are resolved in the current chicken reference genome GRCg7b with other published reference genomes varying between 3-19 D-gene copies. Further, studies in other vertebrates also describe the D-gene region as copy-number variable, suggesting a potential association between the number of germline D-genes and the adaptability of the immune system. The present study therefore aims to investigate the global variability of the chicken D-gene region, with the long-term goal of identifying potential global variation that could be valuable for current breeding programs. To explore the genetic diversity of the D-gene region in a broad set of breeds, we amplified the ~10-25kbp long D-gene region in 124 samples of 79 chicken breeds or lines (economically relevant breeds as well as German fancy and local breeds). The amplicons were sequenced using Nanopore sequencing and a custom pipeline was developed to phase and polish the D-gene haplotypes. D-gene sequences were identified by similarity matching to the 16 known sequences. Additionally, for a subset of the samples also the recombined region of the heavy chain was amplified from B-cells and Illumina Sequencing was used to analyze the usage of the germline encoded D-genes. A clustering of the newly identified D-gene sequences identified distinct clusters of D-genes being closely related to Dx, D1 and D2 respectively, while the other genes formed a relatively undifferentiated large cluster. This structure was also seen in the germline arrangement of the genes, with each haplotype starting with a D1, followed by a Dx and a D2 gene. This conserved region was followed by a variable number of genes from the large gene cluster. Overall, the different haplotypes consisted of 4 to 29 D-gene copies with a mode of nine D-gene copies, uncovering a broad global variation. This exemplarily highlights that chicken genetic resources offer a broad reservoir of unique diversity, which is potentially relevant for an adaptable immune system. Nevertheless, the direct link between the diversity of the D-gene region and the adaptability of the chicken immune system needs to be investigated by further research.

Genomic consequences of a historical bottleneck after demographic recovery in the Old Norse sheep

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Population bottlenecks can have long-lasting consequences for genomic diversity, patterns of inbreeding and the distribution of deleterious mutations, even in populations that later recover numerically. The Norwegian Old Norse sheep is one of the oldest sheep breeds in Scandinavia and belongs to the group of North European short-tailed sheep including Icelandic, Faroese and Soay sheep. The present population of old Norse sheep is considered a direct descendant of the ancestral sheep type present in Norway prior to the introduction of modern breed classifications and unlike many modern breeds, the breed has not been subject to intensive selection for production traits, but rather for individuals capable of thriving outside under extensive grazing systems throughout the year. This has resulted in a hardy sheep adapted to rough grazing environments, characterized by efficient resource use, easy lambings and a double-coated fleece with natural wool shedding. During the mid-twentieth century the population experienced a severe demographic bottleneck, followed by substantial numerical recovery in recent decades. Today the breed is no longer considered threatened based on census size. In this study we investigate the genomic consequences of the demographic history by analysing whole-genome resequencing data from 16 Old Norse sheep AI rams sequenced at >15X coverage. We identify runs of homozygosity (ROH) and partition them by length to distinguish signals of recent inbreeding from historic inbreeding. Most individuals showed low levels of recent inbreeding (FROH ≈ 0.01–0.04 for ROH >8 Mb), although a small number of individuals display elevated levels consistent with recent inbreeding events. Shorter ROHs occur at a relatively higher and similar levels across individuals, reflecting shared historical autozygosity likely associated with the known population bottleneck. Using genome-wide variant annotation, we further investigate whether the demographic history of this population shows genomic evidence of purging of highly deleterious mutations or accumulation of mildly deleterious mutations. Together, these analyses provide insight into how bottlenecks followed by demographic recovery shape genomic diversity and mutation load in managed animal populations and highlight the importance of genomic characterization for the conservation and sustainable use of livestock genetic resources.

Native African cattle as important genetic resource for environmental adaptation

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Native African cattle represent a fascinating genetic mosaic of Bos taurus x Bos indicus, shaped by a complex demographic history and natural selection for adaptation to a vast range of environments. At the core of sustainable agriculture in developing countries is the promotion of locally adapted populations. It is essential to capture the genetic diversity and gain insights into the genetic background that allows native populations to thrive under specific circumstances. Multiple studies have focused on finding selection signatures within and between native African cattle breeds. However, many adaptation traits are associated with the genetic composition rather than breeds per se, i.e. the proportion of indicine and taurine backgrounds, where breeds with a higher taurine proportion have been described as more trypanotolerant while indicine animals are better adapted to drought and heat stress. In this study, we employed different methods to detect selection on ancestral backgrounds (i.e., high taurine or indicine proportions and hybrids), based on whole-genome short -reads sequence data from over 40 breeds from West, Central, East, and Southern Africa. We identified various signals of ancient selection with the respective methods. Results from this study may inform breeding for genetic backgrounds with relevant adaptation mechanisms.

Comparison of genomic vs pedigree-based inbreeding in the Italian Simmental cattle breed

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Inbreeding is a relevant element of the genetic structure of a livestock population, that has to be considered for its genetic improvement and management of genetic diversity. Inbreeding calculation has been traditionally based on the pedigree (INB_PED). The outbreak of genomics has made available tools for calculating the genomic inbreeding (INB_GEN) based on metrics derived from molecular markers. In this work, the pedigree-based and different measures of genomic inbreeding are compared in Italian Simmental breed cattle. A data set of 5,817 primiparous cattle genotyped with the illumina 50K beadchip were analyzed. After edits, 42,100 SNP were retained for further analysis. The following metrics of inbreeding were calculated: the pedigree based (FPED) calculated with a recursive method; genomic relationship matrix based inbreeding coefficient using observed (FGRM) or fixed at 0.5 (FGRM0.5) base allele frequencies; whole genome runs of homozygosity (ROH) based inbreeding coefficient (FROH) calculated with different minimum length classes (2, 4, 8, 16 Mb). Inbreeding depression for milk yield was calculated with a linear model where the EBV was fitted with a random effect of the herd-year combination and the fixed covariable of the inbreeding metrics. The FPED showed a moderate correlation (0.44) with the FGRM0.5 and quite large (>0.55) with ROH-based metrics except FROH16. FGRM0.5 was highly correlated with all FROH measures (>0.75), whereas correlations of FGRM with all other metrics were lower. Principal component analysis (PCA) extracted 4 components able to explain 96% of the original variability. The first (71% variance) was associated mostly with FGRM0.5 and FROH metrics, the second (12%) with FGRM, the third (8%) with FPED, and the fourth (5%) mostly with FGRM0.5 and FROH16, respectively. Factor analysis showed partly similar results. Inbreeding depression estimates were larger for FROH based metrics, with a tendency to increase as the minimum length of ROH included in the inbreeding calculation increased. Pedigree-based and genomic inbreeding show a large degree of concordance and they can be considered together for having a more comprehensive picture of the inbreeding. ROH based metrics allow for estimating separately recent and remote inbreeding.

Detection of homozygosity and heterozygosity rich regions on German Black-Headed Mutton x Kıvırcık (Bandırma) sheep population

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Runs of homozygosity and heterozygosity (ROH and ROHet, respectively) are widely considered to reflect selection pressures and population balances in different livestock species. Investigating these regions is important for shedding light on historical selection and balancing events in populations. In the present study, a total of 391 Bandırma sheep were genotyped using the 50K Affymetrix Ovine Array and mapped onto the "Ramb. V3" reference genome. Following the quality control pruning process, a total of 274 unrelated animals with 40,299 variants were used for further investigations. In terms of detection methods, the model-free method was applied using "DetectRUNS" for both homozygous and heterozygous states. Additionally, QTL enrichment analyses were performed using the "GALLO" package and "animalQTLdb" in R, detecting a total of 7,633 and 904 regions for ROH and ROHet states, respectively. Among these regions, ROH islands on chromosomes 2 and 3 of the ovine genome (OAR-2 and OAR-3, respectively) were found to be significantly enriched for production traits regarding body weight (padj < 1.00E-22) and body circumference (padj < 0.05), with a shared frequency of at least 10% in the population. In addition to these regions, islands located in OAR-9, OAR-18 and OAR-20, which represented 10% of the population, were found to be significantly enriched in exterior (padj < 0.05), health (padj < 0.05) and wool (padj < 1.00E-7) traits, respectively. Investigating the ROHet regions, the OAR-2 ROHet region was significantly enriched for body weight (padj < 1.00E-16), which could be considered a balancing region for body weight on OAR-2. Three additional ROHet regions were identified as QTL-enriched regions. These were located on OAR-8, OAR-3 and OAR-6 and were found to be enriched in wool traits (padj < 1.00E-15; padj < 0.01) and health traits (padj < 0.05). In addition to these results, the genomic inbreeding and diversity coefficients were observed to be 0.0740 ± 0.02880 and 0.0018 ± 0.00087, respectively. Thus, this study provides promising insights into the heterozygous and homozygous states of regions in the ovine genome.

Associations between individual-specific genetic correlations and gametic variability

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Understanding the genetic relationships between traits in dairy cattle systems is critical for the optimal selection of candidates. For example, the genetic antagonism between milk production and conception rate has been widely reported and studied, as such genetic relationships that present a trade-off between traits requires particular attention in breeding programs, to avoid that selection for a trait has a negative effect on the other. This trade-off is quantified by genetic correlations that are negative, and typically estimated as a populational parameter. Such a measure, however, is limited in their ability to comprehend this genetic trade-off at the individual level. While genetic correlations at the populational level have been an effective information to manage trade-offs on a populational context, it ignores the individual's potentially different capacities to manage such joint management of the traits. Recent studies have shown indeed that genetic correlations, including the negative correlation between production and fertility traits in dairy cattle, may differ among the many sires in the selection nucleus. These studies have speculated that such differences in the genetic correlations expressed by different sires may be the partial expression of physiological traits responsible to jointly regulate such traits. Furthermore, aside of potential physiological effects on the expressed genetic correlations between traits, studies on genetic trade-offs between traits usually ignores the recombination of alleles caused by crossovers in the chromosome during meiosis, which will have an effect on the gametic covariances. Thus, two genetically similar sires can pass down different genetic covariability between traits, which can be hard to scale. Our study aimed to associate gametic diversity to the different levels of genetic correlations expressed by ~1500 different sires in a dairy cattle population, for which individual-specific genetic correlations (iSGC) were previously calculated. These sires were genotyped with the Illumina Bovine SNP50 BeadChip (50k SNPs), and for each sire, their phased genotypes were recombined into gametes. Each gamete was created . Finally, the variability of the gametes' variances was compared to the sire's iSGC. This gametic variability was calculated on the whole 50k SNP-genotypes, and on only the SNPs that were targeted as significantly associated with the iSCG obtained between milk yield (MY) and cow conception rate (CR), traits that presented a populational genetic correlation of -0.15. The variability of the gametes specifically on the SNPs that were significantly associated with the iSGC, showed a correlation of 0.1 with the iSGC themselves. This correlation value was approximately twice as large as the one between the gametic variability on the whole genome, suggesting that recombinations at these genomic sites are more relevant, and more strongly associated with observed iSGC differences. Previous studies have already shown that gametic variability depends on heterozygosity at the QTL, and on the linkage disequilibrium between them. Our results indicate that while recombinations explains a part of the differences in iSGC, they still do not explain all their differences, suggesting that other genetic/physiological mechanisms could be responsible for these differences in capacity for trade-off management, which should be studied with further studies, so that the inclusion of these effects can be optimally accounted for in selection and breeding programs, thus allowing better management for genetic trade-off and further sustainable livestock systems.

Analysis of β-casein and β-lactoglobulin variant frequencies in Podolica beef cattle

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Podolica cattle is an autochthonous Italian beef cattle reared in Southern Italy and valued for its rusticity and adaptability to extensive farming systems. Although exclusively bred and selected for beef purposes, its milk is commonly used to produce the typical Caciocavallo cheese on which studies are being conducted to characterize its chemical and microbiological properties, aimed at applying for Protected Designation of Origin certification. Nonetheless, the relatively small size of the breed, the rusticity of the farming system, and its selection schemes that require most of the produced milk to be used for the calf, make Podolica's genetic characterization of milk proteins still unexplored. Among the proteins related to cheesemaking, β-casein constitutes up to 45% of the total casein count, while β-lactoglobulin is the major whey protein in milk. The aim of this work was to genetically characterize the allelic variants of such proteins in Podolica milk. Variants were characterized by SNP genotyping of 208 Podolica cows via medium density SNP arrays (55 or 100K); population genetic values such as allelic and genotypic frequencies, observed and expected homozygosity, fixation index (FIS), effective allele number (Ne), polymorphic information content (PIC), and eventual deviation from Hardy-Weinberg equilibrium were assessed for both loci. Alleles A1, A2, B, and I were observed for β-casein, with allelic frequencies of 0.37, 0.59, 0.02 and 0.02, respectively. Regarding genotypic frequencies, no BB, nor BI genotypes were observed, the observed homozygosity resulted of 50%, and no deviation from Hardy-Winberg equilibrium was detected. β-lactoglobulin showed a relevant majority toward the B allele, with a frequency of 0.86 compared to the A variant, an observed homozygosity of 78% and no deviation from Hardy-Winberg equilibrium. FIS values were negative and close to 0 for both genes, suggesting genetic equilibrium and absence of population substructure, Ne resulted of 2 for β-casein and 1.3 for β-lactoglobulin, while PIC was moderate for β-casein and lower for β-lactoglobulin. These results suggest the presence of favorable alleles for cheesemaking, however the substantial equilibrium of alleles confirm that milk production is not the main aptitude of the breed. Further studies are needed to investigate allelic variants in other milk proteins, and to understand the possible impact of different genotypes in milk chemical and technological traits.

Genetic diversity and effective population size in the Belgian Landrace pig: insights from SNP genotypes and pedigrees

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Monitoring genetic diversity is essential in small livestock populations, where genetic drift and unbalanced sire use can rapidly increase inbreeding and compromise long-term viability. Belgian Landrace, also called Belgisch Negatief Landras (BN), is a local pig breed in Belgium that currently comprises approximately 70 active breeding sows and has a breeding history strongly shaped by selection for stress resistance. Stress susceptibility is associated with poorer technological meat quality (PSE-related traits) and compromised animal health and welfare. Therefore, interest in breeding stress-negative animals has remained high. Here, we provide a genomic and pedigree-based analysis of diversity and effective population size (Ne) in BN using a cohort genotyped with the Porcine GGP 50K SNP array. The dataset included 184 BN pigs (42 boars, 142 females) sampled across 10 farmers, including one artificial insemination station. After quality control, 34,406 SNPs were retained. Genotype completeness was high and SNP markers were informative (median MAF=0.3025). Population structure analysis via PCA suggested modest stratification. PC1 and PC2 explained approximately 21% of total variance and the PCA plot showed no clear subpopulation clustering. Pairwise genomic relatedness indicated widespread coancestry, with 5,530 pairs in the 0.05–0.125 bin, 2,202 in 0.125–0.25, 660 in 0.25–0.5, and 165 pairs ≥0.5, highlighting practical constraints for avoiding close-kin matings. Mean observed heterozygosity was 0.40 and expected heterozygosity averaged 0.38, indicating that genetic variation persists but is unevenly distributed among individuals. Linkage disequilibrium (LD) decay supported a constrained effective size, with R^2 declining to ≤0.2 at ~350 kb and to ≤0.1 at ~1.65 Mb. LD-based Ne increased with time depth (median Ne ≈21.1 at ~8 generations, 35.7 at ~50, and 59.7 at ~100), indicating a smaller recent Ne relative to deeper history. Pedigree-based Ne declined from 46–47 in 2020–2021 and 45 in 2022 to 33 in 2023, consistent with increased recent coancestry. Our results provide a quantitative update based on broader genomic sampling to support monitoring of the breed. Overall, raised genomic relatedness, extended LD and low recent Ne emphasize the need for conservation oriented management in BN. Priorities include kinship aware mate allocation and balancing genetic contributions of boars and herds, especially in AI semen distribution systems. In addition, Ne and relatedness should be monitored routinely using a pipeline consistent workflow to evaluate management impact over time. Ongoing work will compare BN with other Landrace lines (e.g., French and Dutch) using frequency based (FST, θπ) and haplotype-based scans to identify divergent regions contributing to BN distinctiveness and to inform conservation decisions.

Phenotypic data to assess the resilience potential of Sicilian cattle breeds in the context of climate change

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Heat stress is a major limiting factor for animal welfare and productivity in livestock systems, especially in Mediterranean areas. Local cattle breeds raised in challenging climates may show different physiological adaptations to high temperatures compared with specialized breeds. Precision livestock farming technologies enable real-time monitoring of physiological and phenotypic parameters, offering new opportunities to study cattle responses to thermal stress. This study assessed the physiological response to different climatic conditions in two native Sicilian breeds, Modicana (MOD) and Cinisara (CIN), reared in extensive systems, in southeastern Sicily integrating IoT sensor data with hematological and hormonal analyses. Wearable IoT sensors integrated into neck collars continuously recorded heart rate (HR), skin temperature (T_skin) and tri-axial acceleration in MOD (n=13) and CIN (n=12) cows. Trials were conducted in the thermoneutral (February) and hot (July) seasons. Each experimental period included 20 days of monitoring, with blood samples collected at the beginning and end for hematological analysis. The Temperature Humidity Index (THI) was calculated from meteorological station data. Animal activity was expressed as an hourly index derived from accelerometer data. Linear mixed models for repeated measures evaluated the effects of breed, season and their interaction, with THI as a covariate. Overall, 13,330 post-QC HR and T_skin records were collected hourly using wearable IoT sensors hourly for 20 days per season. Heart rate showed significant effects of season and breed, with higher values in winter than in summer (72.2 vs 67.4 bpm), and overall higher HR in MOD than in CIN (74.9 vs 64.4 bpm). Skin temperature was significantly higher in summer, with a significant breed × season interaction: MOD showed higher T_skin values than CIN during summer (37.4 vs 32.8 C°). This suggests different heat-dissipation potential between breeds. Complete blood count results showed significant seasonal and breed × season effects on total white blood cell count (WBC), with higher values in summer in both breeds. Neutrophil counts were higher in winter, particularly in CIN. Lymphocyte percentage was higher in CIN, especially in summer, possibly reflecting a stress-coping mechanism. Eosinophils were higher in MOD and in summer, whereas basophils and monocytes did not vary significantly. Absolute counts confirmed these trends, with the highest neutrophil counts in winter and the highest lymphocyte counts in CIN regardless of season. CIN showed higher red blood cell (RBC) and hematocrit (Hct) values than MOD, particularly in winter. Hemoglobin was higher in winter in both breeds, with peak values in CIN. Erythrocyte indices showed breed differences, with higher mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) in MOD, while mean corpuscular volume (MCV) was slightly higher in winter. The endocrine profile was also analyzed. Cortisol showed only breed-related differences, with higher mean values in MOD. Thyroid hormones (T3 and T4) showed a significant breed × season interaction, while insulin did not differ between breeds or seasons. Overall, results indicate seasonal modulation of hematological traits and breed-specific physiological responses to thermal conditions. This study was carried out within the Agritech National Research Center and received funding from the Next-GenerationEU (PIANO NAZIONALE DI RIPRESA E RESILIENZA (PNRR)– MISSIONE 4 COMPONENTE 2 INVESTIMENTO 1.4 – D.D. 1032 17/06/2022 CN00000022).

Challenges for the Conservation of Farm Animal Genetic Resources under Emerging Animal Diseases

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Infectious diseases constitute one of the greatest threats to the genetic resources of farm animals, leading to declines in population size, reduced breeding value, and the loss of valuable functional traits. In recent years, animal genetic resources in Poland have been under significant pressure due to the occurrence of infectious diseases. Particularly severe impacts have resulted from outbreaks of African swine fever (ASF) and highly pathogenic avian influenza (HPAI) or bluetongue disease. At the same time, the risk of other major epizootic diseases, such as foot-and-mouth disease, remains present. Outbreaks of highly contagious diseases may result in the mass culling of herds, which significantly reduces the genetic diversity of populations. Diseases and the measures taken to control them also affect the implementation of breeding and conservation programmes. Particularly vulnerable are local and endangered breeds, which are characterised by small population sizes and limited security in gene banks, making the recovery of populations after epidemic losses more difficult. Mainstream breeds are generally characterised by larger population sizes, a wider geographical distribution, and better preservation of biological material in gene banks and artificial insemination centres. However, the occurrence of infectious diseases in large, highly specialised populations may lead to significant production losses and disruptions in food supply chains. An additional risk factor is the concentration of herds in a limited number of breeding centres. For some breeds, the number of herds is small and their location is concentrated in only a few places. In the event of an outbreak of an infectious disease, this may lead to the simultaneous loss of a large part of the population. Moreover, populations included in conservation programmes are often maintained in relatively small herds, which limits their ability to recover quickly after potential losses. Within genetic resources conservation programmes in Poland, 89 breeds, lines or varieties are maintained in 3,335 herds, comprising more than 120,000 females. Among them are, for example, laying hens (13 breeds, 19 herds, 12,563 females), geese (14 breeds, 15 herds, 4,726 females) and ducks (10 breeds, 10 herds, 4,152 females). Gene banks maintain a limited collection of semen, mainly from cattle, and a small collection of embryos from cattle and pigs. Some endangered populations are maintained in public stations; however, the majority remain insufficiently secured. Current regulations ensure that information on herds included in conservation programmes is communicated to veterinary authorities; however, mechanisms for rapid feedback and clearly defined procedures in the event of threats to these populations are lacking. To reduce the risk of losing farm animal genetic resources, systemic measures are needed. These should include the development of early warning and rapid information exchange systems between institutions responsible for genetic resource conservation, breeders and veterinary services, as well as the establishment of clear procedures for responding to epizootic threats and the creation of backup herds for the most endangered populations. It is also essential to strengthen the preservation of biological material in gene banks for both local and key commercial breeds that play an important role in livestock production. Such measures would help safeguard valuable genetic resources while also supporting the stability of livestock production and overall food security.

From concept to measurement: Advancing resilience analysis in small ruminant farming systems

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Small ruminant farming systems in Europe operate under accelerating and overlapping pressures derived from global change. Market volatility, policy reforms, labour shortages, shifting societal expectations, and climate-related disturbances are reshaping the conditions under which sheep and goat farms remain viable. These farms function as tightly interconnected socioecological systems, where economic performance, land and feed resources, labour organization, and farmer agency co-evolve. Understanding resilience in this context requires going beyond the narrow logic of "can animal and farm performance be further optimized?" to examine how farms, farmers, and farming communities interact and respond to challenges by withstanding shocks (robustness), adjusting practices (adaptability), or reorganizing their systems more fundamentally (transformability). Over the past decade, resilience has become a central concept in farming systems analysis and policy arenas, yet key gaps persist. First, resilience is often addressed conceptually, with limited translation into operational and comparable indicators at the farm level. Second, resilience assessments have traditionally focused on structural attributes and adaptive capacity at the farm level, often overlooking farmers' psychological resources, despite increasing evidence that job discomfort, and life distress are critical bottlenecks for long-term viability. Third, many empirical studies rely on static assessments or on farmers' perceptions, despite resilience capacities being inherently dynamic processes. Finally, analyses conducted at aggregated sectoral scales tend to overlook the contextual specificities of different farms, production orientations and territories. This invited talk synthesizes results from a set of complementary studies developed in recent years on Spanish small ruminant systems (meat sheep, dairy sheep and dairy goat). Together, these studies move from conceptual operationalization to dynamic quantification and explanatory analysis of resilience capacities. They combine longitudinal quantitative data, farm-level structural indicators, econometric and dynamic modelling, participatory approaches, and survey-based assessments of farmer perceptions and psychological resources. As a whole, this body of work represents a transdisciplinary approach to resilience analysis. By integrating socioecological theory, economic modelling, and farmer-centred perspectives, it highlights both the strengths and weaknesses of existing resilience methodologies. Rather than favoring a single lens, it demonstrates how different approaches capture different dimensions of resilience and why combining them is essential to obtain a comprehensive understanding of the limits, trade-offs and synergies, and development pathways of small ruminant farming systems.

Using extended lactations to shape resilience of goat farming systems: a modelling approach

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Prolonged and repeated heat waves or the arrival of new vector-borne diseases due to climate change have detrimental effects on livestock farming systems. In dairy goat farms, increased culling due to reproductive failure can lead to a decline in herd size and milk production, therefore resulting in economic losses. Historically, dairy goat farmers were using extended lactations (EL) to manage reproductive failure and keep non-pregnant females in the herd. EL consists in maintaining lactation for 485 days or more without intermediate gestation. Dairy goats are able to produce milk for two years and more, not only with a prolonged persistency but also with a pattern of lactation dynamics showing a rebound in production. This practice in dairy goat herds is increasingly used (more than two-thirds of French goat herds) and now concerns not only females that fail to reproduce, but also females that are intentionally not bred. In heat stress situations leading to potential poor fertility results, using EL can maintain overall and reducing the proportion of goats bred, thus mitigating the impact of poor fertility on the herd. This practice also reduces the number of gestations and thus the risky phase associated for the goats and the number kids to cull. There is currently a lack of knowledge on the role of EL in building resilient dairy goat farming systems. There is a need to quantify the consequences of integrating EL in herd management in both perturbed and unperturbed environmental conditions. It is also essential to determine how EL can be integrated into the production system, in line with the farmer's objectives and management practices, particularly by defining the optimal proportion of goats managed in EL. The objective here was to extend the existing Herd Demography Model (HDM) by integrating the management events accounting for two types EL and individual phenotypic trajectories (daily milk production and body weight), based on real data obtained from 84 dairy goats in EL at the MoSAR unit. HDM is an individual-based and dynamic model, which allows to simulate daily physiological stage and milk production of each female in a herd, throughout their lifetime. The modularity of the model allows to implement various breeding practices and reproductive performances. EL can be either unvoluntary in case of reproduction failure, or voluntary when a goat is not bred. For this latter case, three methods can be used to choose goats: random draw, best producers in the herd, or best producers among the goats not selected for artificial insemination (AI). The method and goats' proportion in voluntary and unvoluntary EL are used as model inputs. To illustrate how the model enables to assess the role of EL in dairy goat herds' resilience, a case-study representative of a typical French dairy goat farm was simulated over 20 years (289 goats, with 93 replacement does, and a breeding period from September to November). Two contrasting scenarios were compared. The first one was an unperturbed scenario, with high fertility every year (success rates: 80% AI, 90% natural mating (NM)). The second one was a perturbed one, mimicking a heat stress event occurring in the tenth year at the time of reproduction, leading to a drop in fertility (success rates: 30% AI, 50% NM). For both scenarios, four proportions of goats managed in EL were tested: 0%, 20%, 30%, and 40% of the productive herd. The analysis of the output results provides benchmarks to determine to which extent EL enables to maintain herd demography and production following a sharp decline in fertility.

The Aegean sheep breed revisited: Phenotypic description and husbandry characteristics in island flocks of Greece

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The Aegean sheep breed was once considered one of the most widespread indigenous sheep populations of Greece, traditionally distributed across the islands of the Aegean Sea. Historically described in the literature as the "sheep of the Aegean". The population includes animals from islands such as Samothrace in the north, to Ios in the south, among others. The population was considered to exceed 100,000 breeding ewes in the 1970s. The breed is characterized by strong adaptability to harsh island environments, limited feed resources, and extensive grazing systems, making it particularly suited to Mediterranean pastoral conditions.The ENIPRO project aims to characterize genotypic and phenotypic variation in island sheep populations and to verify the continued existence of the Aegean sheep breed as a distinct genetic population. Within this framework, the present study focuses on the phenotypic description of sheep and management practices Aegean islands' flocks. A total of 43 ewes, originating from flocks located on the islands of Agios Efstratios, Kythnos, Kea, Naxos, Ios, and Sifnos, identified by farmers as belonging to the Aegean sheep breed and maching the description available, were morphologically characterized (mean±SE). Ewes were on average on their 5th lactation (5.04±1.266), with mean prolificacy of 1.46±0.418 lambs per ewe and mean Body Condition Score of almost 2 (1.93±0.418). In terms of ear size, which is described as one of the most distinctive characteristics of the Aegean breed, most animals' ears were horizontal and of noticeable small size, with a mean length of 11.38±0.48 cm and mean width of 6.85±0.382 cm. Head shape was predominantly narrow, of medium size (mean width of 13.83±0.566 cm and length of 25.28±0.469 cm) with a straight profile. Tails were semi fat with an average length of 33.69±2.573 cm. Mean body length was 61.35±1.18 cm and height at withers reached 68.33±1.04 cm. Most of the evaluated ewes were polled (79.06%), while the average horn length for the horned ones was 39.17±4.894 cm. Regarding fleece characteristics, all animals had coarse, white wool and only 18.60% were covered in the abdominal region. Black or brown spots on the head, ears, muzzle, legs and hoofs were observed in all ewes. Skin pigmentation was evenly distributed, with 50% of the ewes exhibiting pink skin and 50% black skin. The udder was cylindrical and well-formed in all animals and the teats were pointing slightly forward, which was recognized by farmers as the most desirable type for suckling. From a husbandry perspective, most farms were small (45-60 animals) to medium (100-150 animals), with Agios Efstratios flocks beeing the only exception with an average size of 638 animals. All farms were family-operated and low-input, relied on limited natural grazing resources, seasonal pasture use and were supplemented with imported concentrates. Limited use of mechanical equipment and intensive housing facilities were highlighted, in consistence to island pastoral systems. Few major health issues, such as mastitis and diarrhea in lambs were reported, reflecting the resilience of the animals under semi-extensive management. These adaptive traits, combined with satisfactory annual flock milk production (mean of 13.068±1.336,61 tn) and reproductive performance under low-input systems, indicate the potential role of such a breed in sustaining traditional sheep farming in the insular agroecosystems of Greece. This research is implemented under the National Recovery and Resilience Plan 'Greece 2.0', funded by the European Union – NextGenerationEU.

Role of territorial stakeholders in the management of risks arising from interactions between wildlife and pastoral livestock systems

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The return and expansion of wildlife in the Alps, particularly large carnivores and large herbivores, are modifying the balance of mountain areas. These developments constitute a major challenge for pastoral systems, which traditionally rely on the extensive use of natural environments and on coexistence between human activities and biodiversity.In this context, interactions between wildlife and livestock farming generate sanitary, social, environmental, and predation risks. The management of these multiple risks relies on institutional and multi-stakeholder governance, placing coexistence-related risk management at the core of pastoral sustainability issues.This study aims to analyze, through the perspectives of the interviewees, how territorial stakeholders intervene in the management of wildlife-related risks within pastoral systems in Europe: the case of the Vanoise National Park in France and the case of the Paneveggio Pale di San Martino Natural Park in Italy. It also seeks to characterize the stakeholders involved in risk management. 33 semi-structured interviews (25 for France, 8 for Italy) and 14 street interviews (only for France) were conducted, using a territorial approach, with a diversity of economic, institutional and social stakeholders, as well as territory users. The analysis of the qualitative data collected follows a thematic approach.Four types of risks (environmental, predation, sanitary, and social) are identified by the interviewees. However, according to the respondents, predation is the dominant risk arising from the interaction between wildlife and pastoralism, with direct and indirect impacts. Nevertheless, economic impacts (direct losses, costs related to herd protection, or modifications of pastoral practices) and psychological impacts (stress, mental burden, questioning of the pastoral system, and farmers' sense of isolation) are strongly emphasized by the respondents. Moreover, the social impact of predation, although indirect, occupies a central place in the discourse: land-use conflicts, misunderstandings between stakeholders, and views of a gap between public policies and on-the-ground realities.Regarding the management of these risks, this work highlights a complex multi-level governance system involving a plurality of stakeholders whose roles sometimes overlap. In addition, the multiplication of levels of intervention leads to confusion regarding the role of each stakeholder, thereby fueling misunderstandings and conflicts.Although management measures are implemented, they are sometimes seen as fragmented, poorly coordinated, and ill-adapted to territorial needs. These views are emphasized by the divergent interests of the various stakeholders, thus reinforcing the difficulties involved in implementing shared solutions. Beyond the current risk management measures currently in place, this study suggests that greater consideration of the social, cultural, and relational dimensions of Human-Nature interactions is necessary. Improving mechanisms for consultation, communication processes, and recognition of local knowledge appears essential to fostering a sustainable and socially acceptable coexistence between pastoralism and wildlife in a context of global change.

Drivers of abandonment risk in autochthonous livestock systems: preliminary results from Castilla-La Mancha.

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The conservation of livestock genetic resources is closely linked to the persistence of farmers maintaining purebred breeding systems. However, the European Union is currently experiencing a decline in the number of farms rearing purebred livestock, reflecting ongoing structural transformations in the agricultural sector. Farmers' exit from the system results from a complex interaction of structural, economic, and behavioural factors, making the continuity of breeding activities particularly vulnerable in marginal and extensive production systems. Understanding the drivers of farm abandonment is therefore essential for designing effective strategies aimed at conserving livestock genetic resources and supporting rural The conservation of livestock genetic resources depends on the persistence of farmers maintaining purebred breeding systems. However, the European Union is experiencing a decline in farms rearing purebred livestock due to structural changes in the agricultural sector. Farmers' exit results from structural, economic, and behavioural factors, making breeding activities particularly vulnerable in marginal and extensive systems. Understanding the drivers of abandonment is therefore essential for designing effective conservation and rural development strategies. The results indicated that behavioural and motivational variables play a key role in farmers' intention to abandon purebred livestock farming. In particular, farmers' professional identity was strongly associated with breed conservation and, therefore, with their intention to continue livestock farming. The second most influential factor was the subjective norm, particularly the component related to support from the professional environment, highlighting the important role of institutions and breeders' associations in the conservation of autochthonous livestock breeds. In addition, factors related to the sector's economic expectations, the perceived socio-environmental value of the breed, and the perceived capacity to continue farming activity were also found to be significant. These findings suggest that the decision to abandon purebred breeding goes beyond purely technical or business considerations, highlighting the emotional and professional attachment to the breed, which creates a sense of belonging and commitment that may act as a buffer against rural abandonment. However, this commitment may be weakened by subjective norms that depend directly on the level of support provided by institutions and the professional environment. When the efforts of farmers are not recognised or validated by their professional and social surroundings, the risk of abandonment increases. Therefore, it is essential to design strategies that promote the social and ecosystem value generated by autochthonous breeds in rural territories, thereby reinforcing rural identity and contributing to the prevention of rural abandonment.

Resilience of European Livestock Systems: multi-scale, context-specificity and methodological insights

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European livestock farming systems are exposed to multiple and interacting challenges, including climate events, market disruptions, demographic transformations and societal pressures. At the same time, they are not merely food production units; they also generate and depend on ecosystem services, sustain rural livelihoods and support cultural traditions. Resilience can be defined as the capacity of a system to resist, adapt and, when necessary, transform in response to disturbances, in order to continue producing food and other services. Resilience is often relative to particular disturbances and defined objectives (resilience of what to what?). This property manifests differently across levels of analysis (e.g., animal, farm, value chain, landscape, region) and each scale makes some aspects more visible than others. Disturbances may be perceived differently according to the geographic region and livestock system type (e.g., monogastrics vs ruminants). Previous studies have advanced in the comprehension of how to measure and asses resilience using both quantitative and qualitative approaches; yet, the multi-faceted nature or resilience – combined with the complexity of European livestock systems – continues to pose challenges to research and policy-making. The purpose of this presentation is to open questions about methodological challenges in assessing and improving resilience of European livestock systems. Drawing on systematic reviews, the presentation highlights how complexity of farming systems influences the way their resilience can be interpreted and evaluated. A systematic review across French regions, combining expert interviews and grey literature analysis, revealed significant differences in how climate-related stressors are perceived across value chains and territories. For example, monogastric sectors showed strong path-dependencies linked to housing infrastructures not adapted to recent climate events, while ruminant systems were primarily constrained by climatic negative impact forage production and land-use dynamics. A review of academic literature highlighted interactions between food self sufficiency and other factors, such urbanisation, water and land use. Urbanisation reshapes rural labour availability and food demand, while water and land resources are shared across multiple competing sectors. These cross-sectoral dynamics show that livestock resilience cannot be analysed in isolation from broader socio-technical and economic environments. These findings suggest that assessing and improving resilience of European livestock farming systems poses methodological challenges: (i) combining quantitative indicators with qualitative approaches capable of capturing actor perceptions across value chains and in different geographic contexts; (ii) conducting context-specific and value chain–specific analyses (no one-size-fits-all approaches), fostering interdisciplinary collaboration, participatory research and cross-sector integration; (iii) applying multiple scales of analysis for both quantitative and qualitative methods. These insights highlight that assessing and enhancing resilience of European farming systems remains a complex and evolving challenge, requiring continued research, stakeholder engagement and methodological refinement.

INOSYS "farm models": a national living lab to simulate change and explore adaptation pathways in French livestock systems

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European livestock farming systems face increasing uncertainty due to climate change, volatile prices and evolving societal demands, which calls for robust empirical bases to assess resilience and support adaptation strategies. In France, INOSYS "Réseaux d'élevage" is a long-standing farm network that annually follows up about one thousand herbivore farms situated in main production areas and in a large diversity of farming systems. The monitoring feeds a national database (DIAPASON) with harmonised structural, technical, economic, environmental and labour indicators. From these data, more than 250 "farm models" ("cas-types") have been built as consistent, data and human-based representations of frequent farming systems in given regions, each combining land use, herd structure and management, input use, production results, work organisation and multi-performance indicators. These farm models are used as a flexible tool to simulate different types of changes, such as climatic variability (e.g. droughts affecting forage yields and grazing) or economic stress (e.g. changes in feed or energy prices), to quantify impacts on technical results and farm income and to identify room for manoeuvre and feasible technical adaptations. Over the 2021–2027 period, INOSYS has been further extended through 18 thematic networks focusing on specific transition issues, including "Adaptation to climate change", "Feed and protein self-sufficiency" and "Cost-efficient systems", which deepen the analysis of adaptation strategies with groups of volunteer farmers. Beyond biophysical and economic resilience, the device has also produced studies on the transmissibility of farming systems (e.g. for generational renewal), workload and the attractiveness of different livestock models, thus linking resilience to farmers' working conditions and willingness to remain in or enter the sector. Overall, INOSYS operates as a national living lab that combines long-term farm monitoring, construction of farm models, change simulations, and thematic co-innovation with farmers and advisers, providing a unique platform to inform policymakers, advisory services and education on the resilience of French livestock farming systems.

Intensive holm oak plantations as a climate adaptation strategy to enhance resilience of traditional Iberian pig production systems

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Interannual variability in natural acorn production has always been a characteristic feature of the dehesa ecosystem, but climate change is intensifying this variability, increasingly challenging the natural resource-based fattening of Iberian pigs during the montanera phase. The Spanish Quality Standard for Iberian pig production requires animals to achieve a minimum weight gain of 46 kg during this phase, feeding exclusively on natural resources from the dehesa ecosystem, to qualify for the acorn category. When acorn availability at the end of the fattening period is insufficient for all animals, some fail to meet this requirement and must be reclassified into categories with lower market value. This reclassification represents a 23% reduction in price per kilogram of live weight, translating to substantial economic losses for producers. This study evaluated whether acorns from intensively managed and irrigated holm oak (Quercus ilex) plantations could provide a complementary resource when natural acorn availability is insufficient, enabling at-risk animals to meet quality standard requirements whilst maintaining production characteristics. A total of 85 purebred Iberian Retinto pigs entered the montanera finishing phase under traditional conditions. After 48 days, 10 animals that were not on track to meet the minimum requirements (weight gain 41.05 ± 0.50 kg; body weight 143.2 ± 1.98 kg) and 10 animals showing adequate progress (weight gain 46.5 ± 0.67 kg; body weight 147.3 ± 1.11 kg) were selected for the study. The underperforming group was supplemented with 10 kg/day of acorns from intensive plantations (established in 2018, average yield 1,500 kg/ha under drip irrigation), whilst the adequate-growth group continued under traditional montanera as controls. Both groups completed the same 63-day finishing period at identical stocking density (0.75 pigs/ha). Acorns from intensive plantations showed comparable nutritional composition to natural acorns. The underperforming group, initially at risk of not achieving the minimum requirement, exhibited significantly higher average daily gain during the final 15-day period following supplementation (1.347 vs 1.224 kg/d; P=0.001), successfully meeting the required 46 kg weight gain and avoiding disqualification. Final live weights were comparable between groups (163.4 vs 165.7 kg; P=0.625). At slaughter, carcass characteristics including weight, yield, backfat thickness, length, and prime cut yields showed no significant differences (P>0.05), demonstrating that supplementation maintained normal carcass development and commercial value. This approach prevented reclassification of all supplemented animals, avoiding economic losses equivalent to 23% of animal value, which represents a critical difference for farm economic viability. Importantly, this strategy is designed as a complementary support tool implemented only during years of insufficient natural acorn production and targeted specifically at animals at risk of disqualification. In conclusion, acorns from intensive holm oak plantations provide a practical climate adaptation strategy that enhances the resilience of traditional extensive livestock farming systems. This approach enables producers to cope with increasing climate variability whilst preserving the authenticity and sustainability of dehesa-based Iberian pig production, contributing to the long-term viability of this valuable cultural and ecological heritage system.

Multidimensional information system for zootechnics: the case study of buffalo farm and the dark side of outsourcing drivers

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The sustainability of animal husbandry represents a controversial topic that is often associated with narratives focusing on accusatory arguments. The following contribution illustrates a relational model of animal husbandry which describe the multifunctional value of the sector, by providing a richer representation that allows to study its metabolic criticalities. To achieve this result, we adopt the MultiScale Integrated Accounting of Societal and Ecosystem Metabolism approach, a relational multicriteria framework allowing the characterization and evaluation of different metabolic features of the system. The integrated characterization of the system includes biophysical aspects (matter and energy flows), economic variables (monetary flows and investments), and relevant information for informing animal welfare strategies. The accounting method allows to evaluate the metabolic performance of animal husbandry using 4 criteria: (i) feasibility – looking at the constraints imposed by the ecosphere (both resources availability on the supply side and the ability to receive wastes on the sink side); (ii) viability – looking at the characteristics of techno-economic strategies adopted by the system; (iii) desirability – looking at the normative and institutional practices adopted by the different social actors; (iv) externalization – looking at the level of openness the system determining its dependence on external resources, a dependence translating into environmental and social externalized impacts. The 20 farms surveyed had an average usable agricultural area (UAA) of 25 ha, entirely devoted to forage production. Feed autonomy (FA) was determined as the proportion of self-produced DM consumed relative to the total DM intake at the herd level. Additionally, energy feed autonomy (FAe) and protein feed autonomy (FAp) were calculated by multiplying the amount of consumed DM by its energy and crude protein content for both purchased and self-produced feeds. Maize silage, often grown in rotation with winter grasses – either as pure crops or in legume mixtures – is the predominant forage. Regarding herd composition, an average of 337 (±188) animals was recorded per farm, including 116 (±74) young females and 213 (±122) adult females. The average milk yield per cow was 2.395 ± 275 kg. Consequently, the average stocking rate was 19±17 heads per h of UAA. In terms of feeding practices, 35% of farms relied exclusively on hay as their forage source, while the remaining farms used varying proportions of maize silage and hay. Compared to herd requirements, 85% of the forage and 100% of the straw and concentrate feed were purchased externally. As a result, the average FA level was estimated at 15%, ranging from 2.5% in dry herds to 23% in lactating herds. Expressed in terms of consumed energy and protein, FAe was 14%, while FAp was slightly lower at 12%. Our preliminary findings reveal a significant dependence on off-farm feed in dairy buffalo farming, with limited forage self-sufficiency and high stocking densities, indicating that scarce arable land is a major challenge to economic and environmental sustainability.

Supply chain vulnerability in protein reduced poultry nutrition: A scenario approach to amino acid supply disruption in broiler production

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Modern protein reduced broiler nutrition relies on globally sourced industrial amino acids (AA) to formulate balanced diets. This structural dependency creates vulnerability within poultry supply chains. Temporary AA shortages may force feed mills to reformulate diets with lower levels of synthetic AA or even without it, increasing crude protein supply, worsening amino acid balance, and impairing feed efficiency. A scenario was developed to explore the systemic implications of such a disruption for Germany. The reference system represents Ross 308 broilers with a 37-day fattening period and a final liveweight of 2.3 kg. Annual production volumes were derived from national slaughter statistics (631 million broiler chickens) and feed production data (6.5 million tonnes of poultry feed per year; 42% allocated to broilers). The scenario assumes that all feed mills in Germany react similarly to reduced AA availability by reformulating the third feeding phase (grower 2; day 21–30) without synthetic AA. To maintain an approximately adequate supply of essential AA, crude protein levels are increased through a higher proportion of rapeseed meal (RSM) in the formulation. Potential negative performance effects in this phase are represented as feed conversion ratio (FCR) penalties of +2%, +5%, and +10% relative to the standard formulation. To determine the duration of AA shortage required to exert a relevant impact on the RSM market, additional demand corresponding to 1% ("noticeable") and 2% ("considerable") of total annual German RSM use was calculated based on the daily additional demand. Environmental impacts (acidification, climate change, and freshwater eutrophication) were assessed using life cycle assessment (LCA) according to the Environmental Footprint (EF) 3.1 method, including changes in nitrogen excretion resulting from altered protein supply. To determine annual system-level impacts, impact factors per kg liveweight were scaled to the annual affected liveweight mass in German and compared to the reference production. Under the baseline scenario (+5% FCR), additional RSM demand reaches 1% of the annual market after 64 days of disruption and 2% after 129 days. Under a lower performance penalty (+2% FCR), the same thresholds are reached later (67 and 135 days), whereas under a severe penalty (+10% FCR) the 1% and 2% thresholds are reached substantially earlier (60 and 120 days). At the 1% threshold (64 days), annualized system changes amount to +591 kmol H□ eq (acidification), +2,453 t CO_2 eq (climate change), and +5 t P eq (freshwater eutrophication), corresponding to +1.3%, +0.1%, and +0.5% relative to the national baseline. At the 2% threshold (129 days), impacts increase to +1,192 kmol H□ eq, +4,944 t CO_2 eq, and +10 t P eq (+2.6%, +0.2%, and +1.1%, respectively). Acidification and eutrophication increase consistently with higher protein supply and nitrogen excretion, whereas climate change shows comparatively smaller relative system-level changes indicating a higher resilience of this factor to short-term protein-driven feed reformulation. The findings illustrate a measurable supply chain vulnerability in poultry nutrition and highlight the structural coupling between nutritional formulation, feed raw material markets, and environmental outcomes in broiler chicken systems. Strengthening the resilience of poultry nutrition to the scenario of AA shortage may include the diversification of AA sourcing and the establishment of a responsive approach that considers all possible outcomes.

Building a resilient dairy-beef calf: Integrating genetics, early-life management, and producer decision-making

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The global expansion of beef production within dairy systems has resulted in a growing population of dairy-beef calves transitioning from dairy farms to specialized rearing and fattening operations. These calves represent a biologically vulnerable population, and their early-life management and genetic background have profound implications for subsequent health, welfare, and performance. Although transport and commingling are frequently highlighted as critical risk periods, resilience at the time of movement is largely shaped by decisions made during the first days and weeks of life, including breeding strategy and neonatal care. This presentation synthesizes current knowledge on how early-life management and genetic selection influence downstream outcomes in dairy-beef calves. Particular attention will be given to colostrum management and adequacy of passive immunity, early life nutritional strategies, age at transportation, housing conditions, and identification of calves following transport that are at elevated risk for disease. Inadequate transfer of passive immunity and suboptimal neonatal nutrition impair immune competence and growth, increasing susceptibility to enteric and respiratory disease and reducing adaptive capacity following movement. Breeding decisions further influence birth weight, vigor, growth potential, and disease resilience, thereby modifying how calves respond to early-life stressors and environmental challenges. Outcomes in dairy-beef calves are shaped not only by biological determinants but also by human decision-making. Emerging evidence indicates that care of calves destined to be raised off the dairy farm is influenced by economic incentives and perceived ethical obligation, contributing to heterogeneity in early-life management. Addressing these structural and behavioural drivers is necessary to achieve consistent improvements in calf health and welfare. Sustainable progress in dairy-beef systems depends on integrating breeding decisions, early-life management, and producer incentives within a coordinated cross-sector approach that builds biological resilience before calves leave the dairy farm.

Early group housing of female dairy calves: first results of the COLOCAVO project

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The COLOCAVO project aims to generate robust reference data on group housing of dairy calves from the first week of life and to identify practical technical pathways that can be implemented on commercial farms. A participatory action-research design was implemented in four experimental farms (1 Les Trinottières, 2 Trévarez, 3 La Blanche Maison and 4 INRAE-IEPL). According to its own initial situation regarding group housing of calves, each farm implement and test some possible modification. The general objective was to have an oveview of group housing in the four different situations. The first iteration was conducted between September 2024 and September 2025. Across the four farms, calves (Prim' Holstein in three farms and Normande in one) were housed, either from birth or from one week of age depending on the facilities, in groups of 2 to 9 calves. The experimental factors were : 1 pair housing and group stability vs instability (to evaluate the effect of mixing when calves were transfered in groups of 6 at 4 weeks of age), 2 milk-feeding frequency (one feeding vs two to reduce cross-suckling), 3 environmental enrichment using non-nutritive teats vs no enrichment (to reduce cross-suckling) and 4 milk delivery method (to test the use of collective feeder). Calves were fed : 1 bucket, 250 L in 8 weeks, concentrate and straw 2 Collective feeder with teats, 450-500 L fermented milk in 8 weeks, hay+cereal+protein crops 3 bucket with teat, 350 L in 9 weeks, concentrate+hay 4 Milkbar, 500 L in 9 weeks, concentrate and straw. Measurement protocols were harmonized across the four sites. Calves were weighed at minimum at birth, weaning, and 6 months of age. Health events (morbidity and mortality) were recorded throughout the trial. Behaviour was assessed on all farms around milk-feeding events and at mid-day at different calf ages, using video recordings or direct observations followed by scan sampling (animals observed at fixed time intervals) with a shared ethogram. Calf growth rates met the target objectives in all four farms, and the tested practices did not affect growth. Mortality and morbidity were very low, null in some cases, under our experimental conditions. Behavioural monitoring identified common patterns across farms. Resting was the predominant behaviour, accounting for 39–69% of the observation period during the first five weeks of life. Feeding behaviour was the second most frequent activity (13–26% of the observation period). Interactions with the environment—when calves licked, sucked, or sniffed elements of the pen (e.g., barriers, feeding rails, walls, or hutches)—represented 4–19% of the time, depending on the farm and housing conditions. Behavioural changes with age were similar across the four farms, with a gradual decrease in resting and a corresponding increase in feeding-related behaviours from an average age of three weeks. Cross-sucking between calves, mainly observed around milk distribution, remained infrequent (1–7% of the observation period). When this behaviour occurred, we hypothesized that it may be associated with the feeding system and/or changes in housing and these hypothesizes will be tested during the second experimental iteration of the project. In conclusion, these results represent initial reference data in the French condition and generated hypotheses that should be confirmed and refined in subsequent iterations.

Is increasing pellet diameter an effective strategy to achieve the withdrawal of straw (forage) in the fattening of crossbred dairy beef calves under straw (forage) scarcity?

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The increasing scarcity and year-to-year variability in straw availability driven by climate change are prompting the search for alternative forage strategies for fattening bulls fed high-concentrate diets. We hypothesized that increasing concentrate pellet diameter may stimulate chewing activity and, consequently, rumination, thereby partially mimicking the functional role of straw as a forage in preventing digestive disorders such as bloat or rumen acidosis. If increasing pellet diameter can stimulate rumination to a similar extent as the ad libitum provision of non-chopped straw, pellet enlargement could represent a suitable strategy to promote rumination during periods of straw (forage) scarcity. Nineteen recently weaned crossbred dairy beef bull calves (98 ± 1.9 kg of BW; 75 ± 2.7 days of age) were individually housed in hutches with sawdust bedding on a concrete floor at a commercial farm in Montgai (Lleida, Spain) during April 2025. Calves were assigned to one of four treatments combining concentrate pellet diameter (3.5 vs. 8 mm) and non-chopped wheat straw provision (yes/no): 1) 3.5+S (n = 5), 3.5 mm pellet with straw; 2) 3.5 (n = 5), 3.5 mm pellet without straw; 3) 8+S (n = 4), 8 mm pellet with straw; and 4) 8 (n = 5), 8 mm pellet without straw. Feed (concentrate and straw, offered in separate feeders) and water were supplied ad libitum. The trial lasted 14 days. Body weight was recorded at the beginning and end of the study. Individual daily intake of concentrate, straw, and water was measured, and health status, including bloat and fecal scores, was monitored. Data were analyzed using a mixed-effects model including treatment, day, and their interaction as fixed effects, with initial BW as a covariate, and with day as a repeated measure. A significant treatment effect was observed for concentrate intake ($P < 0.01$; 2.70_b, 2.16_c, 3.11_a, and 2.40_{bc} ± 0.192 kg DM/day for 3.5+S, 3.5, 8+S, and 8, respectively). Also, a tendency for treatment by day interaction ($P = 0.09$) was detected, with similar concentrate intake across treatments on day 1, followed by pattern divergences thereafter. When comparing the straw-fed treatments (3.5+S and 8+S), straw intake was not affected by pellet diameter ($P = 0.59$; 0.29 vs. 0.25 ± 0.060 kg DM/day for 3.5+S, and 8+S, respectively). Straw provision increased water intake independently of pellet diameter ($P = 0.01$; 7.43_a, 5.83_b, 7.50_a, and 5.60_b ± 0.649 L/day for 3.5+S, 3.5, 8+S, and 8, respectively). No treatment differences were detected for bloat or fecal scores. In conclusion, straw provision enhanced total feed intake in crossbred dairy beef calves. Among calves provided with straw, 8 mm pellet stimulated higher concentrate intake than conventional 3.5 mm pellet. Moreover, in the absence of straw, 8 mm pellet maintained concentrate intake at levels similar to 3.5 mm pellet with straw. These preliminary results suggest that feeding calves with 8 mm pellet without straw may help sustain concentrate intake during periods of straw (forage) scarcity. Rumination data and long-term studies will further clarify the potential of pellet diameter enlargement as a forage-replacement strategy.

Grass-based or maize-based TMR for young bulls have similar effects on growth and carcass quality but different effects on health

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Two differently composed TMRs were simultaneously compared in an on-farm trial with young bulls slaughtered at 10 months of age. A grass-based TMR (G) with grass silage as the main forage part was compared with a maize-based TMR (M) with snaplage as the main forage source. The two TMRs were prepared fresh daily and fed once daily at a 4.5 m feedbunk area in front of each pen. M consisted of 40% snaplage, G consisted of 28 to 35% 1st and 2nd cut grass silage. The TMRs also included barley, rye, sugar beet pellets and vitamins and mineral mixtures. Based on bi-weekly feed analyses, DM (%), CP, starch, NDF, ash (all g/kg DM), net Energy (SFU/kg DM) were 63, 143, 367, 247, 56 and 1.05 for M and 67, 147, 357, 255, 59, and 1.04 for G. Total feed allowance were recorded for each TMR daily. A total of 2 x 180 male calves, 1/3 Holstein and 2/3 Belgian Blue x Holstein calves were enrolled. Mean age and live weight at start was 130 d and 183 kg for G and 132 d and 182 kg for M. Calves were housed in mixed order in 12 adjacent pens in a straw-bedded finishing barn. Data was analyzed using linear mixed models in R with pen as the statistical unit. DMI was 7.7 and 7.6 kg DM/d, ADG 1.44 and 1.39 kg/d, and FCR 5.66 and 5.62 SFU/kg ADG for M and G, respectively. No statistical differences were found regarding performance data. At the mean age of 315 d, calves were slaughtered pen-wise. Mean carcass weight (234 kg), carcass gain (681 g/d), and EUROP conformation (5.6) did not differ between TMRs. However, more carcasses from G (21%) reached EUROP fatness of 3 compared with M (8%). Furthermore, slaughter remarks related to lungs, liver and total remarks were significantly lower for G than M. The relatively low liver abscess frequency (6.5 and 4.8% for M and G, respectively) suggests that both TMRs produced a similar rumen health. Based on this trial, it is possible to include grass silage instead of the commonly used snaplage in a TMR for fast growing bull calves and obtain similar performance and carcass quality.

Reduction of stocking density during the transport of finishing dairy beef calves to the slaughterhouse: truck thermal microclimate and calves' stress indicators

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The transport of live animals is a critical stage in the beef production chain, during which animal welfare can be compromised by multiple factors, particularly environmental conditions. Exposure to high ambient temperatures can alter the microclimate inside the vehicle, increasing the risk of heat stress and compromising cattle welfare. Therefore, it is essential to understand how ambient temperature and stocking density influence internal truck temperature, the thermal environment to which cattle are exposed, and its impact on their welfare. The objective of this study was to evaluate the thermal conditions inside a livestock trailer under different stocking density scenarios while also assessing their impact on animal welfare. Two simultaneous journeys were conducted using two identical trucks along the same route: one with high density (HD; 38 animals, 1.57 m²/animal) and one with low density (LD; 32 animals, 1.88 m²/animal), corresponding to a reduction of one animal per compartment in the LD treatment. The 350 km journey of 5 h 20 min was carried out from a commercial feedlot in the province of Lleida to a slaughterhouse in Valencia, Spain, during August under high ambient temperatures (HT; mean 32.5 °C; maximum 36 °C; minimum 29.5 °C). Animals were crossbred dairy beef bulls with an average live weight of 559 ± 35.9 kg and 350 ± 9.7 days of age. Transport was performed using a two-deck trailer divided into six compartments. Internal and external temperatures were recorded using iButton sensors (DS1923, iButton®, USA). Each compartment contained four sensors (one in each lateral and two in the roof) and sensors attached to the ear tags of three animals to record temperature at animal height. External ambient temperature was recorded using sensors mounted on both side mirrors of each truck. All animals were weighed, and blood samples were collected to assess oxidative stress parameters (serum ROS, TBARS, and lactate) two days before transport and immediately after slaughter. Following slaughter, carcass pH and dressing percentage were measured. Statistical analyses were conducted in R using lmer. Data was separated in three stages: before resting time, during resting inside the truck, and after resting. Stocking density was included as a fixed effect and compartment as a random effect; baseline values for oxidative stress (two days pre-loading) were added as covariates. No differences were observed in internal truck temperatures between the two densities while the trucks were in motion (average of 32.5 vs 32.9 °C for HD vs LD trucks during the total journey). However, while stationary, the HD truck tended to have higher temperatures at the ceiling level compared with the LD truck (AUC 1.13 vs 1.08 for HD vs LD; P = 0.06). When temperature at animal level was evaluated, the higher ceiling temperature was not observed at animal level (1.36 vs 1.34 of AUC for HD vs LD; P = 0.13). The truck density evaluated herein did not affect serum oxidative stress parameters or carcass pH. In summary, during periods of high ambient temperatures, reducing stocking density by 16% had minimal effects on the truck's internal environment and did not positively impact oxidative stress indicators or carcass parameters.

Transport of unweaned calves: risk factors and future perspectives

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The veal and dairy beef sectors are important outlets for surplus calves originating from the dairy industry. The welfare of these calves is a major concern as the animals faces many challenges in the early stages of their life. Among these challenges, transportation is a major player with regard to calf health and welfare. During their production life, calves are moved at a young age from their dairy farms of origin to assembly centres and subsequently to veal or rearing farms. Then calves are transported again at the end of the fattening period to the slaughter house. Transportation includes multiple stressors and critical factors such as handling, commingling with unfamiliar animals, exposure to new environments, food and water deprivation, and thermal stress. Moreover, the first transport moments occurs when animals are young and in their immunity gap period, which makes them more vulnerable to different pathogens. Thus, the combination of all these factors lead to a higher the morbidity and mortality rates in the first weeks post-transport. As a result of the high prevalence of clinical signs (especially related to respiratory and gastrointestinal disorders) there might be a higher use of antimicrobials at the rearing farms, which is a major problem faced by the sector. This presentation will address the most critical factors around transport and their impact on health and welfare of calves, and will provide an overview of future perspectives to improve conditions of surplus calves.

Comparison of organ development at arrival in transported dairy beef calves from two origins: multi origin marketed calves and calves sourced directly from proximity dairy farm

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Marketing, handling, and transportation of unweaned dairy beef calves negatively affect their feed intake recovery and growth, which aligns with the energy deficit and impaired gastrointestinal function typically observed upon arrival at the farm. To develop effective—primarily nutritional—recovery strategies, a deeper understanding of how this commercialization process influences gut and other metabolically relevant organs like liver, thymus, heart and lungs development is required. For this reason, two distinctly different commercialization pathways were compared: multi-origin marketed calves (MO), which experience several transport events and temporary stays in concentration centers—exposing them to stress from loading, unloading, mixing, and variations in both original and center-specific nutritional programs, and proximity-sourced calves, collected directly from nearby dairy farms (DIR), which are not mixed with animals from other origins and share the same initial nutritional program. To conduct this study, a total of 48 calves were used (age between 14 and 30 days). 24 calves of the 48, arrived after being marketed and transported from Germany (MO; initial BW = 60.9 ± 3.02 kg); the other 24 calves were proximity calves that arrived directly from a close dairy farm (< 100 km) (DIR; initial BW = 42.6 ± 3.02 kg). Each group of calves entered at the rearing farm in two batches separated 3 weeks a part. Upon arrival BW and ultrasound score for each calf was recorded to be distributed to be euthanatized in 3 different days (8 calves/treatment/day): d0 after arrival, d7 and d28 to evaluate the digestive tract development. Calves were allocated in collective pens (2 pens/treatment) equipped with an automatic feeder. Calves were fed 2 L of milk replacer twice daily (125 g/L) and ad libitum concentrate feed but were fasted overnight before killing. In the present abstract preliminary data describing the gut empty weight of different sections (rumen, omasum, abomasum, duodenum, jejunum, ileum, cecum, colon) and liver, thymus, heart, and lungs will be described. Data were analyzed using a mixed-effects model including treatment, day, and their interaction as fixed effects, with initial BW as a covariate. Rumen weight tended (P = 0.09) to be smaller in the DIR calves compared with the MO calves throughout the study (227, 225, 744, and 441, 431, 916 g ± 9.61 for d0, d7, and d28, respectively); however this tendency (P =0.11) changed when corrected by BW (0.70, 0.67, 1.29, and 0.53, 0.50, 1.17 ± 0.128 % for d0, d7, and d28, respectively). Abomasum weight corrected by BW was lesser (P = 0.05) in DIR compared with MO calves (0.63, 0.67, 0.62, and 0.73, 0.76, 0.70 % ± 0.0418 for d0, d7, and d28, respectively); contrary duodenum weight tended (P = 0.10) to be greater in DIR than in MO calves (0.15, 0.28, 0.30, and 0.12, 0.17, 0.26 ± 0.494 g for d0, d7, and d28, respectively). Colon weight corrected by BW tended (P = 0.09) to be greater in DIR than in MO calves (0.78, 0.95, 1.20, and 0.79, 0.80, 0.94 ± 0.10 %, for d0, d7, and d28, respectively). Liver weight tended (P = 0.09) to decrease in DIR compared with MO animals where liver weight tended to increase with days after arrival (2.36, 2.12, 2.18, and 1.94, 2.38, 2.32 ± 0.184 kg for d0, d7, and d28, respectively). These preliminary data suggest that where calves originate may influence their organ development and recovery upon arrival at the rearing farm. Ongoing analyses (enzyme activity, microbiota, gene expression, and total gastrointestinal permeability) will add to this information and help design nutritional strategies to improve recovery and welfare.

Nutritional strategy combining sensory additives and olive bioactives enhances post-transport recovery of dairy beef calves with pneumonia

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Marketing and transportation impair health and welfare of unweaned dairy beef calves, and it is more critical when calves are transported with pneumonia, as their feed intake is low. Previous studies have shown that the use of additives to enhance intake and immunity could help to recover calves after the negative effects of stress, feed restriction and fasting suffered during marketing and transportation. A total of 62 calves (65.7 ± 4.37 kg BW and 35.2 ± 3.84 d of age) with pneumonia (ultrasound score ³ 3) arriving at a commercial farm after marketing and transportation were used to evaluate the effect of mixture of feed additives (FA: comprising palatability enhancers, olive bioactives, and tributyrin) added to the concentrate feed on performance and ultrasound lung score until 1 weeks after weaning. Calves were fed 2 L of milk replacer (MR; 12.5% solids) twice daily from d1 to d21, and on d 21 of the study, calves were partially weaned and only milk replacer was offered in the morning, and on d 28 all calves were completely weaned. Calves had ad libitum access to concentrate feed throughout the trial. The study lasted 35 days. Calves arrived in 4 batches and were allocated to 2 pens/batch and assigned to one of 2 treatments (4 replicates/treatment): calves fed a concentrate without FA (CONTROL; n=31); calves fed a concentrate containing FA (COMBO; n=31). Calf BW and pen intake were recorded weekly. A lung ultrasonography was performed weekly. All data were analyzed with a mixed-effects model, except for the lung ultrasound data, which were analyzed using a cumulative link mixed model. Average daily gain tended to be greater over the entire experimental period in COMBO calves (P=0.07) compared with CONTROL calves (0.76 vs. 0.67 Kg/d ± 0.065 for COMBO and CONTROL respectively). Body weight showed an interaction of treatment by time (P=0.07), where COMBO calves had greater BW on d21 (81.5 kg vs. 79.0 kg for COMBO and CONTROL; P=0.04) and d35 (93.2 kg vs. 90.5 kg for COMBO and CONTROL; P=0.02) compared to CONTROL group. Moreover, average concentrate intake over the whole period did not differ between COMBO and CONTROL calves (0.786 vs. 0.683 kg/d, respectively) however, during the first 14 days after arrival when calves fed COMBO numerically doubled the concentrate intake compared CONTROL (d1-d7: 125 g/d vs 74 g/d, d8-d14: 435 g/d vs 222 g/d for COMBO and CONTROL, respectively). Lung ultrasound lesions were not affected by the additive throughout the study period, however, numerically the percentage of calves with pneumonia lesions from day 7 to 28 was lesser in COMBO than in CONTROL calves. These results highlight the potential of using sensory additives and olive bioactives to improve the performance of calves with pneumonia at arrival at the rearing farm.

An overview of dairy-calf systems for meat production in north-west Europe

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The objective of 'Better-Calf', an Interreg North-West Europe (NWE) funded project, is to support rural territories and family farms by encouraging adoption of grass-based dairy calf systems. This paper will provide an overview of the first task on the Better-Calf project; to review current meat production systems using dairy calves in NWE. In all regions represented in this project, Holstein-Friesian is the main dairy cow breed ranging from ~95% to ~50% of total dairy cows in Ireland and Switzerland, respectively. The next most important dairy breeds tend to be dual-purpose breeds more traditionally associated with the respective regions. For example, Montbeliarde and Normande in France, Brown Swiss in Switzerland and Fleckvieh-Simmental in Germany. Crossbreeding dairy cows with beef sires to improve the meat production potential of progeny has increased with the greater availability and use of sexed semen being a key factor in this regard. For example, the use of sexed semen in Swiss dairy herds reached nearly 60% in 2024. In France, beef crossbreeds increased from 15% in 2014 to 27% in 2024. In Ireland, 63% of calves born in the dairy herd in 2025 were beef crossbreds. The main beef breeds used differ greatly with Ireland favouring early-maturing breed types, Switzerland mainly using Limousin and Belgium focusing on the Belgian Blue breed. Systems of meat production range from intensive veal units to more extensive pasture-based beef systems. In Ireland, pasture-based dairy-beef systems predominate contributing 60% of prime cattle throughput. Live export of calves for veal production is also an important market. The contribution of dairy-beef to total beef output in France is lower at ~16% with veal production being the main system for dairy calves accounting for almost 60% of the total. Dairy-beef systems are based on indoor systems for bull beef or pasture-based finishing in the case of steer beef. In Wallonia, dairy calf numbers increased by approximately 30% between 2010 and 2024. Almost all dairy calves bred for meat production are raised in veal systems. The production system is quite integrated with a small number of large companies controlling the production chain. In Switzerland, 45% of dairy calves are raised in veal production systems, 20% are fattened intensively as young bulls, and the rest are raised as heifers and steers in more extensive grass-based systems. Holstein male calves in Germany are mainly finished as young bulls with a large proportion exported for veal production. Progeny from dual-purpose cows, such as Fleckvieh, have greater merit for meat production in either veal or beef production systems. Dairy calves in Luxembourg, excluding heifers needed to replace cull dairy cows, are mainly used for meat production in veal production systems. There are a number of common elements to the development of the dairy-calf meat sector across NWE regions. These include a greater focus on beef-on-dairy, increasing the proportion of forage/grazing in the feed budget and reducing the environmental impact of dairy-beef, mainly in the context of greenhouse gas emissions.

Who Is Willing to Compromise? Consumer Clusters, Societal Acceptance of Alternative Housing Systems and Trade-Offs in German Beef Cattle Production

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BackgroundPasture-based beef production is widely perceived as the most animal-friendly housing systemin Germany, yet remains uncommon in practice due to challenges related to land availabilityand occupational safety. Consequently, pasture-based systems are currently implementedmainly with steers and female animals. Against this background, alternative housing systemsare discussed as potential compromises between societal expectations, animal welfare, worksafety, and structural constraints.ObjectivesThis study investigates (1) societal acceptance of different beef cattle housing systems, (2)public evaluations of key trade-offs in beef production between animal welfare, occupationalsafety, and land availability, and (3) consumer groups differing in their willingness to acceptcompromise solutions relative to pasture-based systems.MethodsData were collected via an online survey in Germany in March and May 2024, applying quotasfor age, gender, and education. After data cleaning, the final sample comprised 965 participants.Respondents evaluated six beef cattle housing systems presented through images and shortdescriptions in randomized order. For each system, overall acceptance and perceived suitabilityas a compromise relative to year-round pasture-based fattening (pasture in summer, housingwith straw bedding and outdoor run in winter). In addition, two trade-off scenarios wereassessed descriptively: (1) pasture-based fattening with increased safety risks versus castrationunder anesthesia, and (2) indoor housing on fully slatted floors under land constraints versusstraw-based alternatives or technical adaptations.A cluster analysis based on perceived compromise suitability is planned. Cluster solutions willbe determined using dendrogram and elbow criteria, with differences examined via ANOVA,post-hoc tests, and cross-tabulations including socio-demographic variables and attitudestoward animal welfare and meat quality.ResultsPreliminary descriptive results reveal pronounced differences in societal acceptance acrosshousing systems. Fully slatted floor systems are largely rejected, with acceptance rates of 8.9%for concrete slats and 13.2% for rubber-coated slats. Willingness to pay premiums for beef from these systems is similarly low (≤6%). Structural modifications within slatted systems, such asopen-front designs, increase acceptance moderately by 17.8%.Straw-based systems receive substantially higher approval. Housing with straw and open frontis accepted by 59.9%, while systems combining straw bedding with outdoor access reachacceptance levels of 75.3%. The highest approval is observed for pasture-based fattening insummer combined with straw-based housing in winter (85.6%).Regarding compromise suitability, slatted systems are rarely perceived as acceptablealternatives to pasture-based fattening (≈15%), whereas straw-based systems with outdooraccess are widely considered viable compromises (up to 70%).In trade-off scenarios, 52.0% favor pasture-based fattening combined with castration underanesthesia to address safety concerns. In contrast, maintaining slatted housing without pastureaccess is largely rejected; instead, 42.9% support conversion to straw-based systems despitehigher costs. Results of the planned cluster analysis are currently under evaluation.

Comparison of two rearing methods for calves: Effects on weight gain and health during the first four weeks of life

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Higher milk intakes and social housing are related to higher body weight gain in calves, as demonstrated in several studies (Welk et al., 2023; Jensen et al., 2015). In some studies, higher milk allowance also resulted in reduced occurrence of disease, but also higher rates of diarrhea were reported (Welk et al., 2023). Social housing supports well-being of calves and is not associated with higher morbidity or mortality (Costa et al., 2016). Hypothesis: The implementation of rearing methods with a higher animal welfare standard for calves is able to improve body weight gain and therefore, could also boost calf health and reduce mortality in dairy practice. Experimental methods: On a dairy farm (950 milking cows), calves were alternately divided in two groups directly after birth: In the control group, calves (n = 69) were tube-fed with 4L colostrum, single-housed and administered with 8L milk/d. In the study group, calves (n = 64) suckled colostrum voluntarily (4.01L), were pair-housed and had a milk allowance of 12L/d. After two weeks, all calves were group-housed but milk allowance remained different. Milk intakes during the first two weeks of life were recorded daily and calves were weighed directly after birth and weekly over the experimental period of four weeks. Treatments according to diarrhea, lung or navel infection or other diseases and mortality were additionally recorded. Statistical analyses: Averaged daily milk intake during the first two weeks as well as body weight gain and treatment frequencies among groups during the first four weeks of life were compared using one-way ANOVA. Results, discussion and conclusion: Social housed calves with higher milk allowance consumed significantly more milk ($P < 0.001$). However, the implementation of more animal welfare related rearing methods did not result in higher weight gain ($P = 0.781$), less treatment rates ($P = 0.551$) or less mortality ($P = 0.18$) during the first four weeks of life. Voluntary intake of colostrum, social housing and higher milk allowance should be mandatory for every calf but improving calf health in dairy practice requires more factors, e.g., hygiene management and vaccination programs, among other things.

Effects of weaning protocols on feed intake and growth performance in calves fed milk replacer ad libitum until 7 weeks of age

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High milk feeding level in preweaning calves results in low solid feed intake. This can lead to impaired growth during weaning when switching to solid feed, because the forestomach system relies on the intake of sufficient amounts of solid feed for the maturation of forestomach digestion. The objective of this study was to identify weaning protocols for calves with ad libitum milk intake during the preweaning period, ensuring tender transition from milk to solid feed intake to secure sufficient energy and nutrient intake for a steady weight gain through the weaning process. Holstein calves (n=36 female and n=34 male) were supplied with milk replacer (MR; 14 % dry matter) ad libitum by an automatic feeder until wk 7 of age. At wk 8, all calves started with 12 L MR/d. Calves were blocked by body weight (BW) and sex and were assigned randomly to one of 4 different weaning protocols. Groups FAST (n=18) and SLOW (n=17) were weaned linearly either within 3 or 6 weeks. Calves in group CON (n=17) were weaned based on concentrate intake. Further reduction of MR occurred when calves consumed 300 g/d concentrates for 3 days. Additional increase of concentrate intake by 100 g/d led to a reduction of 0.5 L MR/d. Calves in group BWG (n=18) were weaned according to average daily gain (ADG) whereas weekly ADG should remain at > 900 g. BW of the calves was determined at each visit using a scale at the automate, and the MR access was reduced by 200 mL/d if the ADG was kept > 900 g/d. If calves in groups CON and BWG had not been weaned by d 105, they were weaned using a linear programme of the automatic feeder until wk 18. Calves were weighed weekly by hand and ADG was calculated up to wk 20. Calves had free access to water, hay and concentrates all the time and to total mixed ration (TMR) based on grass and corn silage from wk 7 on. Daily MR and concentrate consumptions were measured for each visit by the respective automates. In wk 20, daily TMR intake was measured for each calf. Data were analysed by the Mixed Model of SAS with feeding group, time and sex as fixed effects. MR intake was reduced ($P < 0.05$) in FAST and SLOW as planned. FAST showed lowest ($P < 0.05$) MR intake from wk 9 on. MR intake was lower ($P < 0.05$) in SLOW than in CON from wk 10 to wk 14 and in BWG from wk 11 to wk 13. MR intake was lower ($P < 0.05$) in BWG than in CON from wk 12 to wk 14. Concentrate intake increased ($P < 0.05$) during weaning in all groups. Highest intake ($P < 0.05$) was in FAST in wk 10 and 11 and intake was lower ($P < 0.05$) in CON than in SLOW in wk 13. BW was greater in male than female in wk 7 and the effect remained consistent ($P < 0.05$) during treatments until the end of the study (BW in wk 20: 201.6 ± 1.8 kg (male); 176.7 ± 1.9 kg (female)). In female, FAST had lower BW ($P < 0.01$) than BWG from wk 16 to 20. ADG was higher ($P < 0.05$) in male than female calves (ADG: 1.22 ± 0.01 kg/d for male; 1.04 ± 0.01 kg/d for female). TMR intake in wk 20 was higher ($P < 0.05$) in male than in female calves (TMR intake: 12.8 ± 0.4 kg for male; 11.4 ± 0.4 kg for female). No decline in BW development was observed in any of the treatment groups during the weaning period. Weaning in CON resulted in highest MR and lowest concentrate intake during the weaning process. Ad libitum MR intake led to a significant difference in BW between male and female calves before start of weaning and the difference persisted postweaning. The study was supported by the Federal Ministry of Agriculture, Food and Regional Identity (BMLEH, Bonn, Germany), 22-06.01-28-1-C8.07A-21.

Changes of metabolic traits in blood of dairy calves after ad libitum milk feeding using different weaning protocols

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Elevated milk supply enhances anabolic processes and body growth in calves. Metabolic parameters reflect the stimulation of anabolic processes and can function as indicators to identify insufficient nutrient intake during weaning [1]. The objective of the present study was to investigate metabolic changes in ad libitum milk-fed calves during weaning from milk replacer (MR) using four different weaning protocols and to assess parameters that mirror the different weaning protocols. Holstein calves (n=36 female and n=34 male) were supplied with milk replacer (MR; 14 % dry matter) ad libitum by an automatic feeding system until wk 7 of age. At wk 8, all calves started with 12 L MR/d. Calves were blocked by body weight (BW) and sex and were assigned randomly to one of 4 different weaning protocols [2]. Groups FAST (n=18) and SLOW (n=17) were weaned linearly either within 3 or 6 weeks. Calves in group CON (n=17) were weaned based on concentrate intake. Further reduction of MR occurred when calves consumed 300 g/d concentrates for 3 days. Additional increase of concentrate intake by 100 g/d led to a reduction of 0.5 L MR/d. Calves in group BWG (n=18) were weaned according to average daily gain (ADG) whereas weekly ADG should remain at > 900 g. All calves had free access to water, hay and concentrates (wk 2) from first week on and total mixed ration (TMR) based on grass and corn silage from wk 7 on. Plasma concentrations of total protein, albumin, urea, glucose, lactate, beta-hydroxybutyrate (BHB), free fatty acids (FFS) and triglycerides were measured in blood taken before (wk 7), during (wk 8-16 of age), and post-weaning in wk 20 by biochemistry. Data were analysed by the Mixed Model of SAS with feeding group, time, sex as fixed effects. Data on feed intake and growth performance are reported in a companion abstract [2]. Plasma concentrations of total protein and albumin increased ($P < 0.05$) during the weaning process and plasma albumin in FAST was higher ($P < 0.05$) than in SLOW (wk 11 and 13) and BWG (wk 11). Plasma urea concentration increased ($P < 0.05$) with time and was higher ($P < 0.05$) in FAST than in SLOW and BWG in wk 11. Plasma lactate concentration was lower ($P < 0.05$) in FAST than in CON in wk 10. Plasma glucose concentration declined ($P < 0.05$) during the weaning process and was lower ($P < 0.05$) in FAST than in SLOW (wk11), in CON (wk 9-12 and 14) and in BWG (wk 9-12). Plasma BHB concentrations increased ($P < 0.05$) during weaning in all groups, was highest ($P < 0.05$) in FAST from wk 10 to wk 14 and was lowest ($P < 0.05$) in CON from wk 14 to wk 16. Plasma FFS concentration decreased ($P < 0.05$) by time. Plasma FFS in FAST was highest ($P < 0.05$) in wk 10, was lowest ($P < 0.05$) in wk 13 and was lower ($P < 0.05$) than in SLOW (wk 12) and in CON (wk 14). Plasma triglyceride concentration decreased ($P < 0.05$) with time and was higher ($P < 0.05$) in SLOW than in FAST and BWG in wk 11. Changes in BHB and glucose mirrored the weaning process and BHB indicated a greater rumen papilla activity especially in FAST at the beginning of weaning. Higher plasma FFS and urea concentrations in FAST may indicate transient mobilization of fat and protein tissue to compensate for lower nutrient availability due to lower MR intake during weaning [2]. [1] Frieten et al. 2018. J. Dairy Sci. 101:1661–1672.[2] Stoschus A, Diesing L, Nieschulze T, Braun K, Liermann W, Hammon HM 2026. EAAP #1252314441. The study was supported by the Federal Ministry of Agriculture, Food and Regional Identity (BMLEH, Bonn, Germany), 22-06.01-28-1-C8.07A-21.

Effect of weaning duration (3 vs. 6 weeks) on plasma acylcarnitine and free fatty acid profiles in ad libitum–fed dairy calves

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Early-life milk allowance is a key regulator of growth performance and metabolic programming in dairy calves; however, the mitochondrial adaptations accompanying weaning under elevated milk intake remain insufficiently defined. This study investigated the effect of weaning duration (3 vs. 6 weeks) on plasma acylcarnitine and free fatty acid profiles in Holstein calves fed milk replacer (MR) ad libitum. We hypothesized that a gradual 6-week step-down weaning would promote a more stable oxidative transition than a shorter 3-week weaning. Twenty calves were enrolled at approximately 1 week of age, group-housed, and offered MR ad libitum (peak allowance up to 25 L/day) until week 7. At week 8, MR intake was standardized to 12 L/day, after which calves were stratified by body weight and sex and randomly assigned (n = 10/group) to either a 3-week or 6-week linear step-down weaning protocol. Solid feed management was identical across treatments. Plasma samples collected at weeks 10, 13, and 16 were stored at −80°C and analyzed using the Biocrates MxP 500 kit. Data were evaluated using linear mixed models with repeated measures, including fixed effects of weaning strategy, time, sex, and their interactions, and calf as a random effect. Complementary multivariate analyses were conducted via MetaboAnalyst6.0. As designed, calves subjected to the 6-week protocol exhibited a more gradual decline in MR intake and maintained higher milk consumption during early transition. Despite these differences in nutrient supply dynamics, weaning duration did not exert a significant main effect on average daily gain or body weight, although both variables changed over time and differed between sexes. Multivariate analysis revealed marked temporal remodeling of the plasma acylcarnitine profile across the weaning period, with sampling week accounting for the largest proportion of variance. Free carnitine concentrations were influenced by both age and treatment and were higher in calves undergoing gradual weaning during early transition. Short-chain acylcarnitines (C2, C3, C3-DC/C4-OH, C4) were predominantly driven by developmental stage, consistent with maturation of intermediary and mitochondrial metabolism. Treatment-related effects were selective: C5, C5-DC, and C5:1 were elevated in 6-week calves, whereas C6 and C6:1 were greater in 3-week calves; C8 increased transiently following rapid milk reduction, suggesting acute alterations in lipid flux. Most medium- and long-chain acylcarnitines were age-dependent; however, C10:2, C14:2, C16, and C16:1 displayed treatment-specific trajectories favoring gradual weaning during transition. Plasma fatty acids exhibited parallel temporal restructuring, including reductions in several C18–C20 and polyunsaturated species by week 16. Collectively, ontogeny emerged as the dominant determinant of metabolic remodeling during weaning under ad libitum MR feeding, whereas a 6-week step-down selectively modulated mitochondrial lipid-handling pathways and may facilitate a more stable oxidative shift toward a rumen-driven metabolic phenotype.

Prevalence of ESBL/AmpC-E. coli before and after the implementation of specific intervention measures in two dairy farms

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Dairy farms have been identified as a significant reservoir for ESBL/AmpC-E. coli (Gonggrijp et al., 2016). On-farm practices, including antibiotic treatments and hygiene measures in both cows and calves, have been identified as important risk factors in the development and spread of ESBL/AmpC-E. coli (Weber et al., 2021). Hypothesis: The implementation of farm-specific intervention measures on dairy farms, emphasizing disease treatment and general hygiene practices, can reduce the prevalence of ESBL/AmpC-E. coli at farm level. Experimental methods: On two dairy farms (farm 1: 200 and farm 2: 550 lactating cows), fecal samples were collected from animals of all ages and additionally, environmental samples were collected from bedding and feeding equipment. These samples were used to determine the initial prevalence of ESBL/AmpC-E. coli at farm level using bacteriological methods. A questionnaire was used to identify on-farm practices associated with the occurrence of ESBL/AmpC-E. coli. Based on the results of the bacteriological examination and the identified potential risk factors, farm-specific intervention measures were introduced and implemented. The measures included treatment plans for relevant diseases in calves and cows, as well as cleaning and disinfection plans for various housing sections and equipment. Additional sampling was conducted after a one-year period to ascertain whether any alterations in the prevalence of ESBL/AmpC-E. coli had occurred. Statistical analyses: A chi-square test was performed to determine if the implemented measures had led to a statistically significant reduction in the prevalence of ESBL/AmpC-E. coli. Results, discussion and conclusion: The initial conditions were disparate on the two farms, with prevalence rates of ESBL/AmpC-E. coli at farm level being 9% on farm 1 and 41.1% on farm 2. On both farms, the prevalence of ESBL/AmpC-E. coli was age-dependent, with the highest prevalence observed among calves and young heifers, and their immediate environment. Minimal to no occurrence was observed among adult cows. Additional sampling of the farms one year after the implementation of farm-specific intervention measures, revealed a significant decline in the prevalence of ESBL/AmpC-E. coli with a decrease to 1.3% on farm 1 ($P < 0.001$). The reduction to 36.1% on farm 2 was not statistically significant ($P = 0.096$). A potential explanation for the observed differences in the results at farm level could be the higher initial prevalence of ESBL/AmpC-E. coli on farm 2. Given the high contamination levels, it can be posited that a one-year period is insufficient to achieve significant changes in the prevalence at farm level, as the reservoir of antimicrobial resistant bacteria is larger. Furthermore, on farm 2, the implementation of certain measures was inconsistent with the prescribed protocol, constituting an additional explanatory approach for the differing results. The present study showed that the prevalence of ESBL/AmpC-E. coli could be significantly reduced by specific measures that target relevant risk factors regarding ESBL/AmpC-E. coli on farm 1, which had low initial prevalence rates. For farm 2, with higher initial prevalence rates, a reduction without reaching statistical significance could be achieved within one year. A significant decrease of ESBL/AmpC-E. coli prevalence in high contaminated farms probably takes longer. Subsequent studies with a larger sample of farms are required to ascertain the reproducibility of the results and to generate statistically reliable findings.

Lung scanning at arrival of calves in veal calf herds

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BackgroundRespiratory disease is the most significant health challenge in Danish veal calf herds. The aim of this study was to describe the prevalence of ultrasound-detectable lung lesions in Danish veal calves on the day of arrival. Materials and methodsA total of 678 clinically healthy calves from 132 Danish dairy herds were examined by ultrasound on the day of arrival at 7 different veal calf herds. Scanning was performed using a linear probe (Easy Scan) with a frequency of 7.5-MHz. The scans were conducted according to a standardized protocol (qTUS). Calves were scanned on both right and left sides of the thorax from the 6th intercostal space and cranially. Lesions were classified according to qTUS system (score 0 – no lesions; score 1 – consolidations <1 cm ; score 2 – consolidations 1–3 cm; score 3 – consolidations ≥3 cm). Calves with lung scores of 2 or 3 were considered to require treatment and are referred to here as having subclinical pneumonia. ResultsAll 678 calves included in the study appeared clinically healthy at arrival. However, lung ultrasound revealed that a considerable proportion of calves already had detectable lung lesions. Overall, 61% of calves had no detectable lesions, while 39% showed varying degrees of lung changes. In total, 19% of calves had lesions classified as subclinical pneumonia (qTUS score 2–3), despite the absence of clinical signs. The prevalence of subclinical pneumonia varied between visits, ranging from 12% to 30%. Only minor differences were observed in the prevalence of subclinical lung lesions between heifer and bull calves and between different breeds. Likewise, previous treatment for diarrhea or pneumonia in the source herd showed only small differences in the prevalence of subclinical pneumonia. Follow-up data indicated that calves with more severe lung lesions at arrival tended to have higher subsequent mortality and required more treatments for pneumonia during the first 90 days after arrival. Conclusion:The results show that a considerable proportion of Danish veal calves already have subclinical pneumoniaat the time of arrival. These changes would not be found by routine clinical examination. The prevalence of subclinical pneumonia is comparable to that reported in several international studies. Ultrasound examination upon arrival to veal calf herds may contribute to reduced respiratory problems. Early detection makes it possible to initiate targeted treatment of calves with subclinical infections. Early treatment is expected to contribute to more effective therapy, improved recovery, lower mortality, reduced transmission, fewer cases of recurrent pneumonia, and thus overall fewer treatments and reduced antibiotic use. Lung scanning at arrival also makes it possible to identify source herds with particular challenges and thereby opens opportunities for targeted preventive interventions among selected dairy producers. Any future restrictions on antibiotic use may increase the need for lung ultrasound as an early diagnostic tool at the time of calf arrival in veal calf herds.

Normal or low iron level in milk replacers for 1 to 2 months old dairy-beef calves

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Most calf milk replacers (MR) have an iron (Fe) content of 75 to 90 mg/kg. This is much higher levels than found in whole milk. A calf fed moderate to high levels of whole milk will experience a nadir in blood Fe levels around 5-6 weeks of age. When the calf starts to eat concentrate and forage, the Fe content in the feed ration, as well as in the blood, increases. Only a few studies have shown a positive effect of supplying extra Fe to milk-fed calves. Most dairy-beef calves arrive at the fattening farm at 3 to 5 weeks of age. Our research objective was to assess whether a milk replacer with a lower Fe content would be adequate for maintaining recommended hematocrit and hemoglobin levels as well as growth (average daily gain) in calves. Therefore, a standard (S: 78 mg Fe/kg) and Low (L: 30 mg Fe/kg) MR was fed to calves and they also had access to pelleted concentrate and barley straw. Calves arrived in a commercial fattening farm in two batches 2 weeks apart. Within each batch, treatments were assigned randomly to 2 x 2 pens. Thus, a total of 173 calves were housed in 2 x 4 pens each housing 22-25 calves. Calves were 11-54 days of age at start. Pens were straw-bedded. Milk-feeding through automatic feeders continued for 50 days after arrival. Blood samples were taken from 6 calves per pen (n=48) at 5-6 weeks of age to access hematocrit (packed cell volume) and whole blood hemoglobin concentrations. Average daily gain was based on weights at arrival and weight shortly after weaning. Individual veterinary treatments during the study period were recorded. Data was analyzed using linear and mixed models in R with pen as the statistical unit. Average daily gain (800 vs. 805 g/d), blood hematocrit (39.7 vs. 38.0), plasma hemoglobin (8.2 vs. 8.1 mM), and number of treatments against pneumonia (0.95 vs. 1.05) were not different between S and L-calves. All hematocrit-values were in the normal range with only 2 cases between 25 and 30 and 3 high cases between 45 and 52, all not related to the MR-type. All measured hemoglobin levels were above EU required threshold level of 5.3 mM. In conclusion, a low-Fe MR (30 mg Fe/kg) is adequate for maintaining hematocrit and whole blood hemoglobin concentrations as well as health and growth of 1-2 months old preweaned calves offered concentrate and forage.

Alternative forage sources to wheat straw in crossbred dairy beef calves fed high-concentrate diets

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The increasing scarcity and interannual variability of straw availability driven by climate change is prompting the search for alternative forage sources in fattening dairy beef bulls. We hypothesized that several non-conventional forages could replace wheat straw without compromising feed intake. The aim of this preliminary study was to evaluate if in crossbred dairy beef calves three potential alternative forages would have detrimental effects on intake. Twenty recently weaned crossbred bull calves (99 ± 1.2 kg of BW; and 92 ± 1.4 days of age) were individually housed in hutches with sawdust bedding on a concrete floor at a commercial farm in Montgai (Lleida, Spain) during October 2025. Calves were assigned to one of four treatments that differed in the type of forage offered ad libitum, provided in separate feeders from the concentrate: 1) WS (n=5), wheat straw; 2) PN (n=5), panicum; 3) FE (n=5), fescue; and 4) FE+ (n=5), high-quality fescue with greater crude protein content. The trial lasted 14 days. Body weight was recorded at the beginning and end of the study. Individual daily intake of concentrate and forage was measured, and health status, including bloat and fecal scores, was monitored. Forages and concentrate were analyzed for crude protein (CP), ether extract (EE), ash, starch, neutral detergent fiber (NDF) and acid detergent fiber (ADF). Data were analyzed using a mixed-effects model including treatment, day, and their interaction as fixed effects, with initial BW as a covariate, and with day as a repeated measure. Overall and nutrient-specific forage intakes were square-transformed to achieve normality. Concentrate intake did not differ among treatments (P = 0.76; 3.30, 3.09, 3.34, and 3.29 ± 0.253 kg DM/day for WS, PN, FE, and FE+, respectively). Although overall forage intake was also unaffected by treatment (P = 0.22; 0.272, 0.331, 0.409, and 0.316 ± 0.055 kg DM/day for WS, PN, FE, and FE+, respectively), a significant treatment by day interaction (P < 0.01) indicated that calves consumed more FE across on most days, with PN and FE+ showing intermediate values and WS the lowest. Nutrient intake from forage also showed significant treatment by day interactions, and some showed clear treatment effect: forage CP intake was higher in PN and FE+ than in WS and FE (P < 0.01; 16.8, 39.2, 17.1, and 30.5 ± 14.65 g CP/day for WS, PN, FE, and FE+, respectively); forage EE intake was higher in PN and FE+ than in WS (P = 0.01; 4.02, 7.87, 4.40, and 5.97 ± 6.897 g EE/day for WS, PN, FE, and FE+, respectively); forage starch intake was greatest in PN (P < 0.01; 0.30, 1.50, 0.46, and 0.36 ± 2.417 g starch/day for WS, PN, FE, and FE+, respectively); and forage ADF intake was greatest in FE (P = 0.01; 129, 130, 238, and 116 ± 37.7 g ADF/day for WS, PN, FE, and FE+, respectively). In conclusion, the forage alternatives tested in this preliminary study appear to be promising substitutes for wheat straw, with some, like fescue, showing the potential to even increase forage consumption. As the nutrient intake of the alternative forage sources differed from wheat straw, overall nutrient intake was affected. Given the inherent variability in forage composition, longer-term studies are needed to further assess the potential of these forages as viable alternatives to conventional wheat straw in dairy beef calves fed high-concentrate diets.

Performance and Meat Quality of German Holstein Steers in a Silvopastoral System

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Cattle production is a cornerstone of German agriculture, with dairy farming contributing substantially to animal product value. Beyond milk and replacement heifers, dairy systems inevitably produce male calves, whose future remains a major sustainability challenge. This dilemma is particularly evident in organic farming, where only about 10% of male calves remain on their farms of origin, while most are conventionally fattened or exported, practices increasingly criticized for animal welfare, regionality and environmental responsibility. Yet beef from dairy breeds can achieve high quality and may improve climate efficiency. Despite this potential, data on organic, pasture-based fattening of Holstein males of the current genetic are scarce. We therefore investigate silvopastoral grass-based fattening of German Holstein steers under organic conditions as a sustainable and economically viable alternative. The study was conducted at the Thuenen Institute of Organic Farming and included 50 German Holstein steers allocated equally to two experimental groups: pasture with access to a fodder hedge (n=25) or without hedge access (n=25). Animals were raised in two consecutive fattening runs (2023/2024, n =26; 2024/2025, n=24), with balanced group distribution per year. Steers underwent two grazing seasons separated by winter housing. During the grazing periods, animals received no supplementary feed. During winter housing, steers were offered a total mixed ration (TMR) composed of grass silage, maize silage and 1kg concentrate. Animals were weighed weekly from birth until slaughter at 20 ± 1 months. Carcass and meat quality traits were assessed post mortem in the M. longissimus dorsi, including pH (45min, 24h), colour (L*, a*, b*), intramuscular fat (IMF), drip loss, cooking loss and shear force. Statistical analyses were performed in R. Growth was analysed using generalized additive mixed models (REML) including treatment and housing period as fixed effects, age as group-specific smooth term, and animal ID as random effect. Differences in growth curves were evaluated via smooth contrasts. Period-specific daily gains were analysed using linear mixed models. Meat quality traits were analysed using linear models including treatment, year, slaughter time, age and weight as fixed effects. Model assumptions were verified by residual diagnostics. Hedge access had no significant effect on growth or meat quality traits ($p>0.05$). Steers achieved high daily gains with hedge (897 ± 77g) and without (901 ± 67g). Housing significantly affected performance ($p<0.05$), with higher gains during winter housing (1154 ± 171g) than during grazing (861 ± 260g), although pasture gains remained comparable to bulls in similar systems. Slaughter weight and age were within expected ranges. Meat quality was consistently high, with an intramuscular fat content of 4.9 ± 1.6% and low shear force values (53.0 ± 15.6N), indicating very good tenderness. The elevated IMF and favorable tenderness reflect typical steer characteristics and support superior eating quality. Overall, the results demonstrate that pasture-based fattening of Holstein steers can achieve high performance and very good meat quality with limited concentrate use. This system represents a sustainable and welfare-oriented alternative for integrating male dairy calves into regional beef production.

Evaluating short and long colostrum replacer protocols to support recovery in dairy beef calves after transportation

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Dairy beef calves are transported from dairy farms to rearing units, a process that induces stress, triggers nutrient mobilization, and compromises gut health due to fasting. Upon arrival, calves typically show reduced feed intake and growth, along with a higher risk of respiratory disease. Implementing nutritional strategies immediately after arrival may help mitigate these negative effects by promoting early recovery of gut function and feed consumption. Supplementing colostrum—rich in bioactive compounds involved in gut cell development and inflammation—has emerged as a promising approach to support calves during this transition. Commercially available colostrum replacers vary in manufacturing processes and, consequently, in quality. Moreover, the optimal supplementation protocols, including dose and duration, remain to be defined for each product. This is a preliminary study that evaluates two post-arrival colostrum replacer (Calvicol Green, Hokovit, Switzerland) supplementation protocols on performance and feed periods adapt sported dairy beef calves. Ninety-six Holstein unweaned calves were transported from France to Spain in two batches of 48 calves. Upon arrival calves were allocated in individual hutches and distributed by body weight (BW) and lung ultrasonography score to one of the three treatments based on colostrum replacer supplementation: control (CTR) , calves were fed 2.5 L of milk replacer at 125 g/L twice daily; short CR supplementation (SCR), calves were fed 2.5 L of milk replacer at 125 g/L twice daily and were supplemented with 20 g of CR on d0 feeding and 10 g on d1 at the morning feeding; or long CR supplementation (LCR) calves were fed 2.5 L of milk replacer at 125 g/L twice daily and were supplemented with 20 g of CR on d0 feeding and 10 g on d1–3 only in the morning feedings. The SCR supplementation protocol was based on the manufacturer recommendations. Concentrate feed was offered ad libitum. On day 43 calves were weaned and moved to a barn to be allocated in pairs based on their treatment. The study lasted 64 days. Body weight was recorded weekly and feed intake daily. Performance data was analysed in two periods based on housing (d0–43 and d44–64) using mixed-effects models with treatment, period, and their interaction as fixed effects, pen and batch as random effect, and initial BW as covariate. An interaction between treatment and time ($P < 0.05$) was observed in BW, average daily gain (ADG), and milk replacer intake in the first period (d0 to 43). The SCR calves had lower BW and ADG on d 7 after arrival, and lower milk replacer intake on d 7 and 14 after arrival compared with CTR and LCR calves. In the second period where calves were allocated in pairs, only a tendency ($P=0.08$) in ADG was observed among treatments being ADG lower in LCR calves (1.09 ± 0.057 g/d) compared with CTR and SCR calves (1.21 ± 0.057 g/d and 1.26 ± 0.057 g/d, respectively). In this study, the tested supplementation protocol—considering the type of colostrum replacer, the administered amount, and the duration—did not enhance calf performance or feed intake; in some cases, the effects were even unfavorable. Although colostrum supplementation upon arrival remains a promising approach, further research is needed. The quality of the supplement, as well as the dosage and length of supplementation, are likely key factors influencing the ability to support recovery of growth and health after long-distance transportation

Evaluation of the effect of social housing practices for replacement and fattening calves from birth to transport and later stages of life

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Recent studies have shown that early socialization in calves influences behavior, growth, and feed intake both during the rearing period and later in life. While individual housing throughout the milk-feeding period is common worldwide, calves in Europe are usually housed individually only during the first weeks of life before group housing. However, little research has investigated whether social housing in the first weeks provides additional benefits for dairy calves. Moreover, evidence regarding the long-term effects of early social housing remains limited, particularly for dairy calves sold for fattening. This study compares early housing strategies during the first 28 days of life regarding calf health, development, and behavior and evaluates effects in later production stages. The study is being conducted on three German dairy farms with Holstein Friesian and Holstein Friesian × Simmental crossbred calves. Data collection began in June 2024 and will continue until August 2027. Calves were assigned to five housing groups: individual (S28), pair from day 1 (S0P), 7 days individual then pair (S7P), group from day 1 (S0G), and 7 days individual then group (S7G). Calves receive ad libitum transition milk (d 0–10) and milk replacer (d 11–28; 135 g/L). Water, hay, and calf dry TMR are offered ad libitum. During the first 28 days, calves underwent daily health assessments (9 parameters); milk intake was recorded daily, and body weight, umbilical scores, and behavior were monitored weekly or continuously (video). After the 28-day rearing period, female calves are moved to group housing and managed according to individual farm management programs. Male calves are transported to the same calf collection center and then transferred to different fattening facilities. During transport, calves are fitted with heart rate monitors. To assess potential long-term effects of the early housing treatments, weight gain in replacement heifers is monitored, and weight gain in male calves is evaluated throughout the fattening period until slaughter. So far, 147 female Holstein calves, 61 male Holstein calves, and 11 male Holstein × Simmental crossbreds have completed the initial 28-day focus period (S28 = 64; S0P = 56; S7P = 50; S0G = 11; S7G = 30).Based on preliminary evaluations of daily milk intake from one farm, individually housed calves consumed, on average, numerically but not statistically significantly more milk (S28 = 9,172 mL) compared to pair-housed calves (S0P = 8,596 mL; S7P = 8,820 mL).Social housing had a significant impact on average daily gain (ADG) during the first 28 days of rearing (S28 = 981.9 g; S0P = 884.8 g; S7P = 818.2 g; S0G = 674.0 g; S7G = 764.4 g). ADG in S28 was significantly higher than in S7P, S0G, and S7G.Male Holstein calves were slaughtered at an average age of 237 days, with an average carcass weight of 175.2 kg (n = 23). The average daily gain from 4 weeks of age until slaughter was 529.0 g.Overall, preliminary analyses indicate differences among housing treatments; however, a comprehensive assessment must also incorporate health and behavioral data, as well as longer-term effects on subsequent behavior and productivity, which are integral components of this study.

Comparative effects of two sensory feed additives on recovery and adaptation of commercially transported unweaned dairy beef calves from arrival to post-weaning

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Stress and feed deprivation during marketing and transport can impair the early adaptation of unweaned dairy beef calves upon arrival at the rearing farm. The use of sensory feed additives (SA) to stimulate feed intake may support recovery and enhance subsequent performance. Sensory additives may provide olfactory (aroma) and/or gustatory (taste-enhancing) stimulation. This study aimed to compare two SA: one based on a milky aroma (AROMA) and another combining a milky aroma with a sweetener (AROMA+SWEET). A total of 105 Montbéliarde calves (64.6 ± 0.99 kg body weight; 4 weeks of age), commercially transported for 8 h, were monitored for 10 weeks after arrival at a rearing farm. Calves were fed 2 L of milk replacer (MR; 12% solids) twice daily from d1 to d12, 1.5 L twice daily from d13 to d24, and 1.5 L once daily from d25 to d32. Calves were fully weaned on d32 and had ad libitum access to concentrate throughout the study. Animals were allocated to 10 pens (10–11 calves/pen) and assigned to one of two treatments (5 pens/treatment) according to the SA included in the concentrate: AROMA or AROMA+SWEET. Individual body weight (BW) and pen-level concentrate intake were recorded. Data were analyzed using a mixed-effects model. Average daily gain was greater in AROMA+SWEET calves during the pre-weaning period (P = 0.04) and tended to be greater over the entire experimental period (P = 0.10) compared with AROMA calves (+90 g/d and +68 g/d, respectively). Calves receiving AROMA+SWEET had greater BW at weaning (P = 0.04) and tended to maintain higher BW at the end of the trial (P = 0.11) compared with AROMA calves (d32: 91.4 vs. 88.5 ± 1.02 kg; d69: 130.6 vs. 126.0 ± 2.02 kg, respectively). In addition, average concentrate intake over the entire period was greater in AROMA+SWEET than in AROMA calves (2.12 vs. 1.96 ± 0.09 kg/d). In conclusion, supplementation with a sensory additive combining an aroma with a sweetener increased concentrate intake and improved growth performance in dairy beef calves following transport compared with an aroma alone. This strategy may represent a practical nutritional approach to support post-transport recovery and enhance subsequent performance. Keywords: dairy beef calves, sensory additives, post-transport adaptation

Spanish consumer preferences for meat credence signals in beef and lamb: evidence from a mixed logit model

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Consumers are increasingly concerned about sustainability and production practices in livestock systems, which has led to the development of several quality and environmental labels for meat products. Understanding how consumers value these attributes is essential for improving market differentiation strategies in the livestock sector. This study aimed to evaluate consumer preferences for sustainability-related labels in beef and lamb using a discrete choice experiment. A survey-based choice experiment was conducted in which respondents evaluated meat products characterized by different attributes: price, "100% Native Breed" label, environmental traffic-light label, Protected Geographical Indication (PGI) and Animal welfare label. Consumer choices were analysed using mixed logit models to account for preference heterogeneity. Three models were estimated: a model including both meat products and species-specific models for beef and lamb. Results showed that all attributes significantly influenced consumer choice ($P < 0.01$). Price had a negative effect, as expected, indicating sensitivity to price increases. Sustainability and quality-related attributes had strong positive effects. The environmental improvement from yellow to green showed the largest positive effect in the pooled and beef models, whereas the "100% Native Breed" label showed the strongest effect in lamb. Animal welfare and PGI labels also significantly increased the probability of product choice. Overall, results suggest that consumers strongly value sustainability-related attributes, particularly environmental performance and local breed identification. These findings highlight the market potential of environmental, origin and animal welfare labels to enhance differentiation strategies for beef and lamb products.

Better-Calf: Dairy calf systems for resilience, welfare and sustainability in rural territories

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The agricultural sector in north-west Europe is facing several challenges including: (1) economic (e.g. low farm incomes), (2) environmental (e.g. climate change, ammonia, biodiversity decline), and (3) social (e.g. competition for land between producing 'feed' for animal production and 'food' for human consumption, animal welfare, high quality affordable food). These challenges have contributed to a decrease in the number of family farms (e.g. 51% of retiring farmers have no successor in France, 44% in Wallonia), with small farms particularly vulnerable (e.g. 'small' farms in Ireland are reducing at the rate of 1.5% per year). This has implications for rural resilience and the preservation of cultural landscape and biodiversity. Grass-based beef cattle production systems can help to address these challenges. The low cost of grazed pasture when compared to conserved forage such as corn silage and concentrate feeds create opportunities for profitable production systems. Such grass-based systems also imply low competition between human food and animal feed (since livestock are otherwise fed grains which could instead be fed directly to humans), may increase soil carbon storage and improve water quality while also producing local and high-quality food. There is a particular opportunity for livestock farmers to use dairy calves, specifically those not required as replacements in the dairy herd, and to add value to these calves by growing them to mature weights as beef cattle. Presently these calves are often reared in veal production systems. Given ethical concerns with veal systems there is interest in increasing the proportion of dairy calves that are produced in grass-based beef production systems. There are a very wide range of dairy-calf systems that are operated at farm level. Farmers and other stakeholders are often unclear on which system is preferred. The "Better-Calf" project, funded by Interreg North-West Europe, aims to support rural areas and family farms by providing them with tools to optimise grass-fed dairy calf rearing systems. The project will test and promote the implementation of resilient dairy calf systems . Through the support of local and/or national public bodies we aim to create territorial plans for the development of a dairy calf supply chain with optimised management practices. A survey of north-west European citizens and consumers will set out the expectations for the production of dairy-beef and the evaluation of their willingness to pay for those products. Dairy calf systems will be tested on experimental farms with a focus on maximising access to pasture thereby promoting animal welfare. A network of 40 commercial farms across Belgium, France, Germany, Ireland, Luxembourg and Switzerland will be created encompassing a large range of the soil and climatic conditions of north-west Europe. Finally, we will engage with the economic actors in the dairy and beef sectors and public bodies to develop action plans to implement the local production of dairy-beef that maximises returns to all actors and create a more viable sector.

Fresh vs. ensiled Rose (Rosa rugosa Thunb.) pomace: effects on rumen methane production and fatty acid biohydrogenation in vitro

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Rosa rugosa fruit pomace (RFP) is a polyphenol-rich agro-industrial by-product from juice production that could support circular feed systems while potentially modulating rumen fermentation, protozoal activity, methane formation, and biohydrogenation (BH) of unsaturated fatty acids. This study evaluated fresh (RFPF) and ensiled (RFPS) RFP in two in vitro 24-h batch-culture studies (3 independent runs per study) using 400 mg substrate incubated with 40 mL buffered rumen fluid. RFP was sourced from a local Polish processor; a portion was ensiled in 4-dm³ microsilos using a silage additive, and samples were analysed for seed proportion, chemical composition, and phytochemicals. Total polyphenols/tannins were extracted (70% hydrated acetone; ultrasound-assisted extraction), quantified by Folin–Ciocalteu, and profiled by LC–ESI-QTOF-MS. Study 1 compared grass silage (control) with pure RFPF and RFPS. Ensiling increased total polyphenols (465 to 509 mg GAE/100 g DM) and altered the tannin fraction (264 to 228 mg GAE/100 g DM). In pure form, both RFPF and RFPS decreased ammonia, acetate proportion, and methane production (mM/g DM), increased propionate, and reduced the acetate:propionate ratio versus grass silage. Study 2 incorporated RFPF or RFPS into dairy cow total mixed rations at 0, 20, 40, 80, and 100 g/kg DM. Across inclusion levels, ruminal pH was maintained, and IVDMD/IVOMD declined only slightly; the high seed fraction of the pomace (~63%) likely contributed to this modest digestibility response. RFP reduced H□ concentration and decreased total protozoa numbers, but methane yield did not consistently decline up to 100 g/kg DM, suggesting that the effective dose of bioactive compounds was insufficient to strongly affect methanogenesis under these short-term conditions. RFP also modified fatty acid BH in vitro. For fresh RFP, linoleic acid BH decreased (87.0% in control to 83.8–85.0%), and linolenic acid BH showed small reductions at selected inclusions (minimum 87.0% at 80 g/kg). For ensiled RFP, linoleic acid BH decreased at low inclusion (e.g., 82.0% at 20 g/kg vs 86.2% control), while linolenic acid BH varied across inclusions (84.7–90.6%). Overall, RFP (particularly when ensiled) shows promise as a local, low-waste feed ingredient for shifting fermentation toward propionate and reducing protozoa and BH, with only a modest methane-mitigating effect in 24-h batch culture. These results justify follow-up work in longer-term and more dynamic systems (e.g., RUSITEC/in situ) to better resolve dose–response relationships and impacts on methanogens and fermentation stability.

Effect of a combination of hazelnut and cocoa bean by-products on in vitro ruminal fermentation and biohydrogenation

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Hazelnut skin (HZN) and cocoa bean shell (CBS) are agro-industrial by-products, rich in fatty acids (FA) (HZN) and bioactive compounds (phenols in HZN and CBS, and theobromine in CBS), that could be valorised as feed for ruminants, promoting circular economy. Several studies reported modulatory activity on ruminal fermentation and biohydrogenation of individual HZN or CBS in ruminant diets. However, little is known about their possible additive or synergistic effects. This in vitro study evaluated ruminal fermentation and biohydrogenation responses to the addition of a HZN and CBS blend in ruminants' diet. Batch cultures of rumen microorganisms were conducted using rumen fluid from three cannulated ewes. The basal diet was composed (% DM) of grass silage (43), hay (35), and cereal- and soybean-based concentrates (22). Three inclusion levels (0, 1.5, and 3.0% diet DM) of each by-product, alone or as their 50/50 mix, were incubated for 30 h and total gas (GP) and CH4 productions, volatile fatty acid (VFA) yield and proportions were measured. Data were analysed with R software using a linear mixed model with treatment as fixed effect and incubation run as random effect, followed by Tukey's post-hoc test. The GP decreased with the HZN-CBS blend at both the 1.5 and 3.0% inclusion levels (−7% and −8%, respectively). A similar trend was observed with HZN at 1.5% and CBS at 3.0% (–5% and −9%, respectively). Treatments induced minor changes in VFA: a reduction in butyrate proportion and yield (on average, –8%) and proportional increases in acetate and propionate proportions (only significant at the 3.0% of HZN-CBS mixture). The greater supply of cis-9 18:1 with HZN resulted in higher levels of this fatty acid (range: +51 to +70%) and its isomerization products in HZN-containing treatments. Major biohydrogenation pathways (trans-11 and trans-10) were not affected. Inclusion of HZN in the diet increased the content of 9+10-oxo-18:0 (range: +152 to +229%), which derive from an alternative metabolic pathway of cis-9 18:1 metabolism, suggesting variations in microbial function or composition, in agreement with the parallel reductions in major odd- and branched-chain fatty acids. The CBS did not influence biohydrogenation, and no synergistic or antagonistic interaction with HZN emerged. The studied by-products appear as sustainable feed ingredients, with HZN showing additional potential to modify biohydrogenation. Acknowledgement: PIE202540E090 (CSIC, Spain). Horizon Europe AgroServ project n.101058020 (UNITO, Italy).

Effect of carrot inclusion level on the in vitro fermentation kinetics of dairy sheep diets

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Spain ranks among the world's leading carrot producers; however, a significant portion of the harvest is discarded as surplus or cull carrots due to strict commercial standards. Within the framework of the circular economy, upcycling these discarded products as livestock feed offers a sustainable strategy to reduce environmental impact and feed costs. Given their high content of fermentable carbohydrates, the aim of this study was to evaluate the in vitro ruminal fermentation of dairy sheep diets with increasing amounts of carrots. Four isoenergetic and isoproteic diets (40:60 forage:concentrate) were formulated: a control diet (CON) and diets with 8, 16 and 24 % DM of carrots (CAR8, CAR16 and CAR24) replacing part of the concentrate of the CON diet. Three hundred mg of substrate (n=4) were incubated in batch cultures of ruminal microorganisms at 39 ºC, and gas production was measured at 3, 6, 9, 12, 17, 22, 26, 31, 36, 48, 72, 96, 120 and 144 hours. Endogenous gas production was corrected by subtracting the gas volume produced by the corresponding blanks from the total gas produced in the vials. Data were fitted to a nonlinear model to estimate potential gas production (A), fractional rate (c) and lag time. Average gas production rate (AGPR) and effective degradability of DM (DMED) were also calculated. Gas production values were fitted to the model: $Gas = A(1-e^{-c(t - Lag)})$, using the NLIN procedure of Statistical Analysis System (SAS). The asymptotic gas production was not affected by the replacement of concentrate with carrots (mean 72.6 mL/g DM; $P > 0.05$). However, increasing the carrot content in the diet led to a marked linear reduction in lag time (L; $P < 0.0001$), which decreased from 4.63 h in the CON diet to 2.15 h in CAR24. The fractional rate of gas production showed a quadratic response ($P < 0.001$), reaching its maximum value with the CAR16 diet (0.062 %/h). The average gas production rate increased both linearly and quadratically ($P < 0.01$), while the effective degradability of dry matter (DMED) showed a linear increase ($P < 0.0001$) as the level of carrots increased. Specifically, DMED values improved linearly from 32.9 % (CON) to 36.7 % (CAR24), indicating that the inclusion of carrots enhanced the rate and extent of the initial ruminal fermentation. The results indicate that the inclusion of carrots significantly enhances fermentation efficiency by reducing the lag time and increasing both the average gas production rate and the effective degradability of dry matter. Under the conditions of this study, a 16% inclusion level (CAR16) appeared to be the most efficient in terms of the fractional rate of gas production, providing the best balance between rapid energy release and microbial processing speed, suggesting that carrots are a high-quality alternative energy source for sheep, capable of accelerating the initial fermentative processes. Funding from the Spanish State Research Agency (AEI) and the European Regional Development Fund (Project PID2021-124648OB-C22) is gratefully acknowledged. N. Merino acknowledges the support of the Junta de Castilla y León for her predoctoral contract.

Rumen degradability, fermentation dynamics, and methane production of potato co-products using in vitro batch culture and rumen simulation technique

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Potato processing generates large volumes of co-products with potential as alternative energy sources in ruminant diets, yet their comparative nutritional value and longer-term fermentation effects remain insufficiently characterized. This study evaluated ruminal fermentation characteristics, nutrient degradability, gas kinetics, and methane (CH4) production when five potato co-products—potato cream (PC), cooked potato chips (CPC), raw potato chips (RPC), potato slice (PS), and potato peel (PP)—replaced wheat grain in a control diet (CTL) formulated with a forage-to-concentrate ratio of 55:45 (DM basis). Three runs of a complementary in vitro batch culture system (72 h; n = 4 per treatment) screened all potato co-products, followed by a semi-continuous Rusitec trial (14 days; n = 4 per treatment) to evaluate the most promising co-products (PC and PS), using rumen fluid from donor cows fed a basal diet at maintenance energy level, with both experiments performed under completely randomized designs. Chemical composition differed markedly among co-products: fibre-rich PS and PP contained approximately 2.5-fold higher neutral detergent fibre than wheat grain, whereas thermally processed PC and CPC exhibited comparable or higher starch concentrations. In batch culture, PC and CPC increased propionate production by 11–23% ($P < 0.001$) and reduced acetate-to-propionate ratios by 15–17% ($P < 0.001$) relative to CTL diet, indicating a shift toward propionate-oriented fermentation, while gas kinetics showed 14–17% faster fractional fermentation rates for PC and CPC ($P < 0.001$), consistent with their higher fermentability and starch availability. Additionally, CH4 yield decreased by 16% with PC and by 30% with PS compared with the CTL ($P < 0.001$). Under Rusitec conditions, PC maintained total VFA production comparable to CTL (120 vs. 123 mM/day), increased propionate production by 14% ($P = 0.001$), reduced butyrate by 25% ($P < 0.001$), and substantially lowered ammonia-N concentrations ($P < 0.001$). Dry matter disappearance was comparable between PC and CTL, although PS showed markedly lower degradability ($P < 0.001$), reflecting compositional differences in fibre and lignin content. Daily gas production was maintained with PC but reduced with PS ($P = 0.045$), while CH4 production showed consistent numerical reductions (12–20%) for potato co-products compared with CTL, accompanied by modest improvements in hydrogen recovery efficiency. Temporal fermentation dynamics showed no significant treatment-time interactions for most fermentation parameters, indicating that the observed metabolic shifts persisted consistently throughout feeding cycles rather than representing transient post-feeding effects. These findings indicate that thermally processed potato co-products, particularly PC, show potential to replace cereal grains in vitro while maintaining fermentation activity and reducing methanogenesis. However, in vivo validation remains essential to confirm these results under practical feeding conditions.

Can corn in a dairy sheep ration can be replaced with cull carrots?

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Spain is one of the main producers of fruits and vegetables in the EU, generating large amounts of horticultural waste that are discarded at different stages of the food supply chain. The use of these discarded products as feed ingredients for ruminants could help reduce both the environmental impact associated with their disposal and the feeding costs of livestock production. Cull carrots represent a potential alternative energy source in ruminant diets. The aim of this study was to evaluate the effect of replacing corn with cull carrots in the diet of dairy sheep on feed intake, live body weight, feed efficiency, and milk yield and composition. Two groups of ten lactating Assaf ewes on the second third of lactation were fed on two different isoproteic and isoenergetic diets (total mixed rations) for 28 days. The control group (CTL) received a total mixed ration ad libitum and the carrot group (CAR) received the same ration but corn was totally replaced by fresh cull carrots (160 g/kg DM) previously chopped in 1 cm pieces. Milk yield was recorded daily and milk samples were taken on days 1, 8, 15, 22 and 28 for chemical analysis: fat, protein, and lactose. Individually feed intake was daily recorded, using two automatic feeders (SmartFeed, C-Lock, USA) per group. Feed conversion ratio (FCR) was estimated by dividing the average daily dry matter intake by the average daily milk yield. Ewes were weighed at the beginning and the end of the trial, to determine body weight change. Total dry matter intake (DMI) and live body weight remained similar between experimental groups throughout the experimental period. No differences were observed between diets in milk yield over the 28-day period (2121 and 1961 g/day for CTL and CAR, respectively; $P > 0.05$), but a significant diet x day interaction was observed ($P < 0.05$). FCR was similar between both groups (1.15 and 1.30 g DMI/g milk for CTL and CAR, respectively; $P > 0.05$). No significant differences were detected for protein, fat or lactose concentration in milk ($P > 0.05$). However, a tendency for a diet x day interaction was observed for milk fat content from the 5 sampling days and was numerically higher in the CAR group (5.58 and 5.85% for CTL and CAR, respectively; $P = 0.05$). The results of the present study suggest that cull carrots can replace corn in the diet of dairy ewes without negatively affecting dry matter intake, milk yield, feed efficiency or overall milk composition during mid-lactation. These findings support the potential use of carrot as an alternative energy source in dairy ewe ration without compromising productive performance. Funding from the Spanish State Research Agency (AEI) and the European Regional Development Fund (Project PID2021-124648OB-C22) is gratefully acknowledged. N. Merino acknowledges the support of the Junta de Castilla y León for her predoctoral contract.

Valorization of agro-industrial by-products: effects of brewery spent grains and olive leaves on milk quality and sustainability indicators in grazing ewes

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Brewery spent grains (BSG) and olive leaves (OL) are agro-industrial byproducts rich in nutrients and phenolic compounds, often discarded as special wastes. Yet, their recovery as a feed ingredients for ruminants may offer both environmental and nutritional benefits. Indeed, polyphenols have gained interest for their ability to modulate ruminal metabolism, potentially improving microbial protein metabolism and milk quality, and the use of agro-industrial by-products could reduce the feed-to-food competition and reliance on imported commodities. Given the above, we hypothesized that the inclusion of BSG and OL to partially replace conventional cereal and pulses in a pelleted concentrate for grazing lactating ewes could influence animal performance, milk composition, coagulation properties, antioxidant capacity, fatty acid (FA) profile, rumen volatile fatty acids (VFA), and human-edible feed conversion efficiency (HEFCE). Twenty-two mid-lactation Comisana ewes were allocated to two homogeneous groups: a control group (CTRL; n=11), receiving 800 g/head/day of commercial pelleted feed, and an experimental group (TRB; n=11), receiving a pelleted feed containing 35% dried brewery spent grains and 7% dried olive leaves. Both groups grazed together for 8 h/day and received 450 g/head/day of alfalfa hay. After a 15-day adaptation period, individual milk yield and samples for chemical analyses were collected weekly over a 30-day experimental period. At the end of the trial, rumen content was collected using an esophageal probe. Data were analyzed using a mixed model to test the fixed effect of diet and sampling time, and their interactive effect (diet ´ sampling time) for performances and milk quality parameters. Diet was included as a fixed effect for VFA data. The animal was included as a random effect. Differences were considered statistically significant when $P \leq 0.05$. Tukey's test was used for multiple comparisons. Animal performance was not negatively affected by the TRB diet ($P>0.05$). Milk urea ($P \leq 0.05$) and somatic cell count ($P<0.001$) were lower in the TRB group, likely due to the higher polyphenol content of the TRB concentrate. Lactose was higher in TRB milk ($P \leq 0.05$) and rennet clotting time and curd firmness were improved ($P<0.001$). Milk total saturated FA decreased, whereas mono- and poly-unsaturated FA increased in TRB milk ($P<0.001$). Specifically, stearic acid (C18:0) increased ($P<0.05$), likely reflecting both a greater dietary supply of this FA and increased ruminal biohydrogenation due to the higher intake of C18:3 n-3 and C18:2 n-6 in the TRB diet. Also, C18:3 n-3 and C18:2 n-6 increased in TRB milk. Moreover, C18:1 isomers and biohydrogenation intermediates including rumenic acid (C18:2 c9t11) increased ($P \leq 0.05$), suggesting alterations in ruminal biohydrogenation pathways. The n-6/n-3 ratio increased for TRB milk but remained within recommended nutritional limits. Atherogenic and thrombogenic indices were significantly reduced ($P<0.001$) in the TRB group compared with CTRL. Milk antioxidant capacity was not affected by diet ($P>0.1$). Propionate concentration in rumen content increased ($P=0.04$), indicating possible alterations in VFA metabolism. The HEFCE improved in the TRB diet. In conclusion, our findings suggest that BSG and OL improved milk technological properties and lipid profile without impairing animal performance. Moreover, the improved HEFCE suggests a potential reduction in feed-to-food competition and a lower environmental impact, supporting the use of these by-products within circular livestock production systems.

Sesame Seed Meal as a Local Alternative Protein Source in Lamb Diets: Effects on Animal Performance and Meat Quality

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The feedstuff market currently faces significant challenges due to price volatility and recurrent shortages of key ingredients. These issues are particularly evident for high-protein feed sources such as soybean meal and other conventional feed ingredients. Consequently, there is increasing interest in identifying locally available alternative protein sources that are affordable and readily accessible, while maintaining animal health and productivity and reducing the environmental impact of feed supply chains. In this context, the present study aimed to evaluate the effects of including sesame (Sesamum indicum L.) seed meal, produced in the Mediterranean area, in lamb diets on animal performance and meat quality. Sixteen male weaned lambs (40 days old; 15.3 ± 0.51 kg body weight) were selected from the farm flock and randomly assigned to two experimental groups. Two isoenergetic and isoproteic diets were formulated. The first group was fed a conventional diet (CON) consisting of maize (43%), alfalfa hay (32%), soybean meal (20%), molasses (3%), and a vitamin–mineral premix (2%). The second group received a similar diet in which 10% sesame seed meal (SES) replaced soybean meal. After 50 days of feeding, all lambs were slaughtered at a local slaughterhouse. Carcasses were weighed and stored at 4 °C for 24 h. Subsequently, samples of the Longissimus dorsi muscle were collected for further analyses. The partial replacement of soybean meal with sesame seed meal did not affect voluntary feed intake ($P > 0.05$). Ruminal pH and carcass characteristics also showed no significant differences between the two experimental groups ($P > 0.05$). Likewise, meat quality traits related to moisture retention — such as water-holding capacity, drip loss, and cooking loss — as well as color parameters, were not significantly affected by diet ($P > 0.05$). The total contents of saturated (SFA), monounsaturated (MUFA), and polyunsaturated fatty acids (PUFA) in intramuscular fat were not influenced by sesame inclusion ($P > 0.05$). However, the concentration of health-promoting fatty acids, particularly vaccenic acid (C18:1 t11) and rumenic acid (C18:2 c9,t11), was substantially increased in lambs fed the diet containing 10% sesame seed meal ($P < 0.05$). In contrast, the SES diet resulted in a reduction in the proportion of odd- and branched-chain fatty acids (OBCFA). In conclusion, sesame seed meal can be considered a promising alternative protein source for lamb diets, allowing partial replacement of soybean meal without negatively affecting animal performance, carcass traits, or meat quality. Moreover, its inclusion improved the profile of health-promoting fatty acids in intramuscular fat. These findings support the potential use of sesame seed meal as a sustainable feed ingredient in Mediterranean livestock systems. This study was carried out within the Agritech National Research Center and received funding from the European Union Next-GenerationEU (PIANO NAZIONALE DI RIPRESA E RESILIENZA (PNRR) – MISSIONE 4 COMPONENTE 2, INVESTIMENTO 1.4 – D.D. 1032 17/06/2022, CN00000022). This manuscript reflects only the authors' views and opinions, neither the European Union nor the European Commission can be considered responsible for them. Part of this research was funded by the projects "Gen.Al.Tec." (Linea 1—PIAno di inCEntivi per la RIcerca di Ateneo 2024/2026; P.I. Andrea Criscione) and "GreenFeed" (Linea 3 Starting Grant – PIAno di inCEntivi per la RIcerca di Ateneo 2020–2022; P.I. Antonio Natalello).

Replacing soybean products with dried brewer's grains and sunflower meal in light lambs' diets: implications on meat quality and fatty acid profile.

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The use of agro-industrial by-products in animal feeding is increasing due to their contribution to farms sustainability and circular economy in the livestock sector, although their impact on the quality of animal products must be evaluated. This study assessed the effect of replacing soybean meal and hulls with dried brewer's grains and sunflower meal in the concentrate of light fattening lambs on meat pH, chemical composition, fatty acid (FA) profile, color and lipid oxidation measured as TBARS (thiobarbituric acid reactive substances) concentration. Twenty-two male Assaf lambs (15.5 ± 0.34 kg body weight) were divided in two homogeneous groups and each was assigned to one dietary treatment. Two iso-nitrogenous (145 g crude protein/kg) and iso-energetic (0.98 units for meat production/kg) concentrates were formulated: a high-cereal concentrate containing soybean meal and hulls (control) and a concentrate (BGSF) in which both soybean products were totally replaced with dried brewer's grains (16.1%) and sunflower meal (16.5%). All lambs were fed ad libitum concentrate and barley straw and were slaughtered at about 25 kg body weight. Meat pH was measured in the longissimus thoracis and semitendinosus muscles at 0 and 24 h post-mortem, and both chemical composition and fatty acid (FA) profile were analyzed in a sample of longissimus lumborum after 24 h of chilling. In addition, changes in color and lipid oxidation (TBARS concentrations) were assessed over 6 days of refrigerated storage in a sample of longissimus lumborum. Two lambs from the control group had health problems and were withdrawn from the experiment. Data on meat pH, chemical composition and FA profile were analyzed by analysis of variance with the fixed effect of concentrate. Meat color and lipid oxidation data were analyzed as a mixed model with repeated measures, in which the effects of concentrate, time (0, 3 and 6 days) and their interaction were considered fixed, and the effect of lamb was considered random. The type of concentrate had no effect ($P > 0.05$) on meat pH, chemical composition (moisture, ash, protein and fat content) and content in total saturated FA and monounsaturated FA. However, lambs fed the BGSF concentrate had greater content of polyunsaturated FA (436 vs. 356 mg FA/100 g meat; $P = 0.034$), indicating a healthier FA profile. Feeding the BGSF concentrate also increased meat content in n-6 FA (384 vs. 307 mg /100 g meat; $P = 0.025$) and the ratio n6/n3 (13.0 vs. 11.6; $P = 0.003$). Concentrate type had no effect ($P > 0.05$) on either color or lipid oxidation of meat over storage and no interaction concentrate x storage time was detected ($P = 0.740$). In conclusion, replacing soybean products with dried brewers' grains and sunflower meal did not negatively affect meat quality traits and slightly improved its polyunsaturated FA content, supporting the use of these feeds as sustainable alternatives in lamb feeding.

Long-term impact of avocado seed (Persea Americana L.) inclusion during early-life on ruminal fermentation, blood parameters, fertility and milk yield performance in Murciano-Granadina goats

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Spain is the leading avocado producer in the European Union [1]. The guacamole industry generates substantial quantities of by-products, with seeds (AS) accounting for approximately 30% of the total fruit weight. [2]. Previous studies by our research group have demonstrated that AS is a high-energy ingredient due to its high starch content with degradation profiles comparable to those of cereals commonly used in ruminant diets [3,4]. However, its use in ruminant nutrition remains controversial due to potential toxic effects. Therefore, it is essential to evaluate its safety and efficacy through in vivo under real production conditions. For this purpose, 32 post-weaning Murciano-Granadina goats (14.2 ± 0.291 kg) were divided into two experimental groups: one control group (CTL) fed with a commercial concentrate, and a treatment group (AVC) fed with a commercial concentrate with an 11% of inclusion of AS (dry matter basis). Blood and live weight were collected from 20 goats per group at 75 (S1), 120 (S2) and 240 (S3) days of age. Additionally, ruminal fluid samples were obtained during the S2 and S3 periods. To characterize the dietary effect on animal productivity, individual milk yield and composition were monitored. Statistical analyses were performed using SPSS and R software. One-way ANOVA was conducted using GLM for single-point measurements and LMM for body weight and blood parameters. Significance was set at $P < 0.05$. No significant differences were found between treatments in animal body weight. Regarding the rumen fluid parameters at S2, the concentrations of isobutyrate (2.60 vs. 1.70 mol/100 mol, $P = 0.002$) and isovalerate (2.83 vs. 1.52 mol/100 mol, $P= 0.001$) were higher in the AVC compared to the CTL. Conversely, the AVC showed lower total short-chain fatty acids concentration (34.3 vs. 51.2 mmol/L, $P = 0.001$) and butyrate molar proportion (11.3 vs. 13.8 mol/100 mol, $P = 0.002$). By S3 sampling, no significant differences were detected in any ruminal fermentation parameters. Regarding blood parameters, no significant main effect of diet was observed, except for total cholesterol (TC), where the AVC treatment showed lower overall levels than CTL. A significant diet × time interaction was revealed for lipid fractions and NEFA. Specifically, both TC and HDL-cholesterol ($P < 0.001$) showed a marked decrease in the AVC group by the S3 period, resulting in lower final values compared to the CTL group. Similarly, NEFA levels displayed a significant interaction ($P = 0.021$), driven by an increase in the AVC group at S2 that was not observed in the CTL group. Fertility rates improved in AVC compared to the CTL (89.7 vs. 65.2 %). While milk yield and composition remained unaffected by the treatment, the somatic cell count was reduced in AVC goats both postpartum (135 vs. 343 10^3/ml, $P = 0.005$) and at the lactation peak (222 vs. 489 10^3/mL, $P = 0.005$). Despite the observed differences in ruminal fermentation, animal growth and milk yield remained unaffected by the treatments. Notably, the AVC diet suggested an improvement in mammary health. Furthermore, the increased fertility in AVC may be linked to the observed reduction in circulating TC, which could stimulate progesterone synthesis [5]. These beneficial effects, coupled with the absence of toxicity, position the AS as a functional and sustainable ingredient in ruminant nutrition. [1] FAOSTAT. 2024; [2] Rojas-García, A. 2022. DOI: 10.3390/antiox11061049, [3] Yáñez-Ruiz et al., 2021. AIDA-ITEA XIX, [4] Martín-García et al., 2021. AIDA-ITEA XIX, [5] Baulland et al., 2021. DOI: 10.5455/javar.2021.h540.

Adapting Dairy Cow Diets Toward a More Resilient and Efficient System by Using Sorghum Silage

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Water scarcity and climate change are constraining dairy production by reducing key feed resources availability, such as corn silage, which—despite its high yield and energy content—is increasingly challenged by drought and mycotoxin risks. This drives interest in more resilient forage alternatives. Sorghum (Sorghum bicolor L.) offers strong drought and salinity tolerance, requires less water and fertilizers, and allows multiple harvests, making it a promising alternative. This study aimed to evaluate the impact of replacing corn silage with sorghum silage in dairy cow diets. A total of 80 Holstein cows (parity: 1-6; body weight (BW): 703 ± 8.4 kg; milk yield: 37.6 ± 0.98 kg/d; days in milk (DIM): 194 ± 9.9 days) located in 4 different pens were blocked by parity, DIM and milk yield, and randomly assigned to 1 of 2 treatments: CS (corn silage–based diet; 18.7% CP, 1.63 Mcal NEl/kg, 30.5% starch, and 25.6% aNDF, on DM basis) or SS (sorghum silage-based diet; 18.7% CP, 1.65 Mcal NEl/kg, 28.7% starch, and 26.8% aNDF, on DM basis). Over 60 d, BW, milk yield and composition were recorded daily by AfiFarm system (V 5.2.1 A-DU). Feed intake (FI) was measured at pen level to estimate feed efficiency (FE). Additional milk samples were collected biweekly and analyzed by NIRS (MilkoScan) for fat, protein, lactose, solids non-fat, somatic cell count, and urea. Data were analyzed using repeated measures in SAS (V. 9.4) considering one-previous week average pre-trial values as covariates and including block effect. No differences in BW or BW change were noted between treatments. Cows in SS tended ($P = 0.08$) to produce more milk than CS cows during the first 5 weeks, with no differences thereafter. Milk AfiLab system data showed that milk fat concentration increased ($P < 0.03$) in SS during the first 5 weeks of study compared with CS cows, whereas fat yield remained greater ($P < 0.04$) in SS than CS throughout the trial, except for the first week. The use of either corn or sorghum silage did not affect milk protein content, but milk protein yield tended ($P = 0.09$) to be greater in SS than in CS cows, mainly during the first 4 weeks ($P < 0.04$), consistent with the greatest milk yield. Milk Milkoscan analysis revealed a greater ($P < 0.01$) milk fat percentage in SS than in CS cows at the 5th sampled week, probably due to the slight differences in starch and fiber contents in the diet. Similarly to AfiLab system data, no variations were detected in milk protein content. However, milk urea concentration was numerically greater in SS than CS cows throughout the study, but significantly greater ($P < 0.01$) at week 4, suggesting an excess of CP in their diet. Feed intake was similar in both treatments except for the 5th week that was lower ($P < 0.05$) in SS compared with CS cows. Following the same trend, FE did not differ between treatments, but it tended ($P = 0.07$) to be lower in CS compared with SS cows during the 5th week. The lower milk yield and similar FI during the first weeks of study in CS compared with SS cows suggest that the adaptation to the new diet for CS cows was more challenging for the digestive system than for SS cows, probably due to the greatest starch content in CS diet. Afterwards, both diets performed similarly without compromising FE or milk quality. In conclusion, sorghum silage represents a resilient alternative to corn silage that maintains dairy cows' performance and milk quality.

Wheat Silage and Hempseed Cake as Sustainable Alternatives in the Feeding of Dairy Cows

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In temperate regions, corn silage and soybean meal are key components of dairy cow diets, due to their high nutritional value. However, climate change, increasing mycotoxin risk, and declining European self-sufficiency are challenging their sustainability and safety. Consequently, interest is growing in resilient alternatives such as autumn–winter forages and oilseed co-products, although evidence regarding their combined use in dairy cows' diets remains limited. This study aimed to evaluate the effects of replacing corn silage with wheat silage and soybean meal with pelleted hempseed cake (Cannabis sativa L.) on productive performance, milk quality, behavioural activity and health in lactating dairy cows. A crossover trial was carried out at the experimental farm of the University of Padova (Italy). Four primiparous Italian Simmental cows (DIM: 148 ± 85; milk yield: 24 ± 5 kg/d) were assigned to two balanced groups (G1 and G2) and housed in individual pens. The study followed a 2 × 2 crossover design over 8 weeks. After a 1-week adaptation phase, cows were subjected to two experimental periods (3 weeks for each) separated by a 1-week washout period to minimize carryover effects. During period 1 (weeks 2-4), G1 received the conventional corn silage-soybean meal diet (CTR), routinely used on the farm, whereas G2 received the experimental diet based on wheat silage and hempseed cake (WSHS). Diets were reversed in period 2 (weeks 6-8). Both diets were formulated to be isoenergetic and isonitrogenous, averaging (% on DM): crude protein 15.05 ± 0.51, neutral detergent fiber 35.86 ± 1.74, starch 19.17 ± 0.75, and 1.47 Mcal/kg net energy. During each experimental period, milk, blood and rumen samples were collected weekly (3 samples per period, 6 per variable overall). Behavioural data were recorded through neck collars (SenseHub™, Allflex), providing 21 daily records per period for each variable. Data were analysed by ANOVA using the MIXED procedure of SAS. Results showed that DMI and milk yield were higher in cows fed WSHS (+2.36 kg DM, +1.38 kg/d; P<0.001), whereas feed efficiency was lower compared with CTR diet (-7.63%; P<0.001). The 4% Fat-corrected milk was not affected by the treatment. Milk fat, protein, and urea nitrogen were not influenced by the diet. Milk from cows fed WSHS had lower SFA (68.12 vs. 69.62%; P<0.01) and higher total PUFA (5.49 vs. 4.19%; P<0.001), including higher PUFA n-6 content (4.80 vs. 3.69%; P<0.001), resulting in a reduced n-6/n-3 ratio (6.98 vs. 7.40; P<0.01). Blood glucose, non-esterified fatty acid, aspartate aminotransferase were not affected by treatment. However, β-hydroxybutyrate was lower in cows fed WSHS diet (0.45 vs. 0.52 mmol/L; P<0.05), while γ-glutamyl transferase tended to increase (24.20 vs 25.10 U/L, P=0.09). Similarly, rumen pH, ammonia nitrogen, and total volatile fatty acid profile were unaffected by diet. Among behavioural traits, overall activity was lower in cows fed the WSHS diet (305.1 vs. 317.6 min/d; P<0.001), whereas rumination and eating times did not differ between treatments. In conclusion, wheat silage and pelleted hempseed cake could represent strategic alternatives to conventional feeds, improving milk fatty acid composition without compromising productivity or health. Acknowledgements: Funded by Ministry of Agriculture, Food Sovereignty and Forests of Italy, D.M.n. 0461776 del 18/09/2024 prot. n. 0544040 del 15/10/2024. Project "DI.L.CA.VE. Valori e autenticità"

Improving the nutritional value of canola, flax, and wheat straw to beef cattle with calcium hydroxide treatment

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Forage production and management remain persistent challenges for livestock producers, where drought and adverse weather events such as hail frequently reduce yields and constrain forage availability. Developing strategies to enhance crop residue utilization offers an opportunity to reduce reliance on traditional forages, both during periods of scarcity and in years of favorable crop production. The objective of this study was to evaluate the effect of treating canola, flax, and wheat straw with calcium hydroxide on dry matter intake (DMI), ruminal pH, the concentration of ruminal short-chain fatty acids (SCFA), fractional rate of neutral detergent fiber passage (Kp) and degradation (Kd), and nutrient digestibility. Six ruminally canulated heifers were used in a 6 × 6 Latin square design with a 3 × 2 factorial treatment structure. Treatments included wheat (WS), canola (CS), or flax (FS) straw included at 40% of the dietary dry matter (DM). Straw sources were either treated with calcium hydroxide (CH) or limestone (CTL) as a control. For treatment, straw was hydrated to 50% moisture, combined with either CH or CTL at 5% of the DM weight, and stored for 24 to 48 h prior to feeding. Experimental periods lasted 24 d, with 14 d of dietary adaptation and 10 d of data and sample collection. Samples collected included feed and refusals, ruminal pH, ruminal fluid, ruminal digesta, and total fecal output. Data were analyzed using the GLIMMIX procedure of SAS 9.4 with fixed effects of straw type, calcium treatment, the straw type × calcium treatment interaction, and period. No interactions between straw type and calcium treatment were observed ($P \geq 0.13$), thus only main effects are discussed. Heifers consuming CH treated straw had 13.4% greater DMI than those consuming CTL treated straw (7.4 vs. 6.5 kg; $P < 0.01$). Dry matter intake was greater for WS and FS ($P < 0.01$) than CS. No effect of CH treatment was observed for ruminal pH ($P \geq 0.41$), but heifers fed CS had the greatest mean ruminal pH and those fed FS had the lowest ($P < 0.01$). Total ruminal SCFA concentration was 13.4% greater for heifers fed CH treated straw over CTL (124.9 vs. 110.1 mM; $P < 0.01$) and was less for heifers fed CS compared to WS and FS ($P < 0.01$). Use of calcium hydroxide increased the NDF Kp from 1.52%/hr (CTL) to 2.1%/hr (CH; $P = 0.02$) with no effect of straw treatment ($P = 0.12$). There was no effect of treatment on Kd ($P \geq 0.35$). Dry matter digestibility increased by 5.4% (57.8 vs. 54.8%; $P = 0.03$) and gross energy digestibility increased by 4.6% (61.4 vs. 58.7%; $P = 0.02$) for CH compared to CTL. A tendency was observed for greater organic matter (OM) digestibility in the CH treatment compared to CTL ($P = 0.08$). No effect of calcium hydroxide treatment was observed for ash-free neutral detergent fiber (aNDFom), or acid detergent fiber (ADF) digestibility ($P \geq 0.22$). The DM digestibility was greater for WS and FS relative to CS ($P < 0.01$). Gross energy digestibility and aNDFom digestibility were greatest in the WS treatment ($P < 0.01$). Acid detergent fiber digestibility was greatest for the WS treatment, intermediate for the FS treatment, and least for CS ($P = 0.02$). Calcium hydroxide treatment increased dry matter intake, total ruminal SCFA concentration, the fractional passage rate of aNDFom, and apparent dry matter and gross energy digestibility, regardless of straw type. Thus, these results suggest that calcium hydroxide treatment could serve as a practical approach to improve straw quality.

From waste to worth: Chemical, physical and functional properties of spent mushroom substrates with implications for ruminant nutrition

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Spent mushroom substrates (SMS) are the residual biomass remaining after mushroom cultivation. SMS uniquely combines two abundant natural polymers namely chitin (a fungal cell-wall component) and lignocellulose from the sawdust growing medium, each with applications in animal nutrition. We characterized the chemical, physical, and functional properties of SMS from shiitake, king oyster, and oyster mushrooms. Thirty-six SMS samples were collected over four consecutive weeks (three replicates per week; 12 per mushroom) from a local producer in Vienna. We assessed chemical composition, fat-binding capacity, antioxidant properties, total phenolic content, and in vitro ruminal degradability. Across mushrooms, SMS was low in crude protein (3–5%) and ether extract (0.1–0.3%) but high in fiber (69–84% neutral detergent fiber) on a dry matter basis. Shiitake SMS showed greater ruminal degradability (58% potentially degradable organic matter vs. 42–43% for oyster and king oyster ($P<0.01$), consistent with its lower lignin content (8.8% vs. 11–14%). It also had higher total phenolic content and antioxidant capacity ($P<0.01$) All SMS bound oil at 2.7–3.2 times their weight, with oyster SMS showing the highest value $P<0.05$). Overall, shiitake SMS outperformed oyster and king oyster SMS on several criteria. These findings highlight SMS as a promising, harvestable resource for applications in animal nutrition, especially for ruminants. There is still the need to address remaining technical gaps and to evaluate feed safety to support decisions on integrating this circular biomass into the food chain.

Exploring the use of fresh apples in dairy cow diets

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Non-saleable fresh apples from proximity orchard could be used in dairy cow diet as a sugar source rapidly digested in the rumen, which may be used by rumen microbes to grow. To explore the use of fresh apples in dairy cow diets, 2 studies were performed. In the first study, a total of 7 primiparous and 7 multiparous lactating Holstein dairy cows (182±87.4 DIM, 27.8±5.51 kg milk/d) were fed either a control diet (15.8% CP, 1.64 Mcal NE/kg DM, CTRL) or a diet with 2 % DM of apples (15.5% CP, 1.64 Mcal NE/kg DM, APP). Feed intake, milk yield and feed efficiency were daily recorded for a 52-d period. During the last week of the study, milk samples were obtained for milk quality analysis, and rumen fluid for 2 consecutive days were sampled to determine rumen pH and pooled for VFA and microbiota analysis. In a second study, a total of 22 primiparous and 52 multiparous lactating Holstein dairy cows (190±98.4 DIM, 30.4±6.86 kg milk/d) were fed either a control diet (16.5% CP, 1.59 Mcal NE/kg DM CTRL) or a diet with 2 % DM of apples (16.3% CP, 1.60 Mcal NE/kg DM APP) for a period of 37 days. Milk yield, feed intake and feed efficiency were daily recorded, and milk samples obtained every 2 weeks. At day 36, rumen samples were collected from 4 cows per treatment and incubated in vitro with their own diet (plus an intermediate diet with half the amount of apple content) for gas production measurements and VFA determination. In study 1, energy-corrected milk, dry matter intake, and feed efficiency were similar in both treatments. Milk composition was similar in both treatments with the exception of milk urea concentration, which was lower ($P < 0.05$) in APP than in CTRL cows (0.24 vs 0.32 ± 0.158 g/L, respectively). Rumen VFA concentrations and proportions were similar in both treatments, except for a trend ($P = 0.08$) of a lower propionic acid concentration in APP than in CTRL cows. Chao1 and Shannon indexes for the prokaryotic community were greater ($P < 0.05$) in the rumen of APP than in CTRL, suggesting a more diverse rumen ecosystem. Taxonomic analysis revealed a greater relative abundance of Bacteroidales and lower Lachnospirales order in CTRL than APP cows, but the overall community composition did not differ across treatments based on a PERMANOVA analysis. In the second study, a decrease was observed ($P < 0.005$) in DMI in APP compared to CTRL cows (24.0 vs 25.5 ± 0.42, respectively) resulting in an improvement ($P = 0.05$) on feed efficiency (1.49 vs 1.43 ± 0.030, respectively). Total gas, methane, CO2 and the concentration of total VFA and individual VFA after in vitro incubation were unaffected by the treatment. Non-saleable fresh apples can be used at 2% of the dairy cow diets without performance impairment and rumen fermentation shift.

Implementation of a short-term palatability test to evaluate dairy cows preference for Carinata meal

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Carinata meal from Brassica Carinata is a high-protein byproduct from extraction of oilseed, representing a sustainable feed ingredient supporting circular agriculture. Its nutritional value is comparable to soybean and canola meals; however, the presence of antinutritional factors such as glucosinolates and a higher content of erucic acid may limit its inclusion in dairy cows' diets by reducing palatability and limiting intake. The objective of this study was to assess the palatability effects of replacing 10% of canola meal with carinata meal in a concentrate, implementing a short-term methodology evaluating dairy cows' preference. Eight rumen cannulated Holstein Friesian cows (229.4 ± 92.1 days in milk; 38.6 ± 6.4 kg/d milk) were housed in tie-stalls and fed a lactating cow diet, formulated for a 740 kg cow producing 32 kg/d of milk. The concentrate included barley (22.8%), high sugar beet pulp (19.0%), canola meal (15.2%), and 7.6% each of corn, wheat, low-sugar beet pulp, and lupin, plus full-fat soybeans (6.8%), soybean meal (3.8%), calcium carbonate (1.1%), and salt (0.8%). Carinata meal contained 90.2% dry matter (DM), 45.6% crude protein, 2.0% ether extract, 8.4% ash, 27.3% neutral detergent fiber, and 29.3 µmol/g DM of glucosinolates. Each cow received 10.4 kg concentrate per day (representing 14.8% of the diet (as fed)), with 4.4 kg mixed into the partial mixed ration (PMR) and 6 kg offered separately in four 1.5 kg portions containing either treatment A (0% of canola meal replaced by carinata meal), treatment B (10% of canola meal replaced by carinata meal), or treatment C (90% of canola meal replaced by carinata meal as negative control). Palatability test consisted in offering the concentrate twice daily for three minutes in paired buckets with randomized left/right positions, reversed between morning and afternoon to control for side preference. To avoid confounding effects of hunger, cows received treatment after receiving their PMR diet. Treatment A and B were offered in the first two experimental days, followed by treatment B and C during the subsequent two days. Feed intake was recorded, and observations of time spent eating and number of visits were recorded by observational scoring. Data was analyzed using mixed effects models (lme4 package in R) including fixed effects of treatment and bucket position and random effects of cow and day. For the positive control (A vs. B), inclusion of 10% carinata meal did not affect feed intake, time spent eating, or number of visits. Bucket position influenced intake ($P = 0.02$), number of visits ($P < 0.01$) and time spent eating ($P = 0.03$) with cows showing a consistent preference for the left bucket. However, no interaction between treatment and position was detected, indicating that treatment effects were independent of bucket position. For the negative control (B vs. C), 90% carinata inclusion reduced intake ($P = 0.02$), time spent eating ($P = 0.01$) and number of visits ($P = 0.01$) relative to the 10% inclusion. Bucket position affected intake, but no treatment and bucket position interaction was observed. These findings indicate that cows differentiated treatments based on palatability rather than positional bias, confirming the functionality of the palatability test used in this experiment. Substituting 10% of canola meal with carinata meal did not reduce palatability when feeding a concentrate, supporting its potential as a sustainable protein source in dairy rations. Future studies should investigate higher inclusion levels and the long-term effects on intake and performance.

Protein value for ruminants of brewer's grains from Spanish breweries

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Brewer's grains (BG) are the main by-product of the brewing industry and, due to their high content in crude protein (CP) and high-degradable fiber are commonly used in ruminant feeding. However, some factors, such as the raw materials and the processing methodology employed in beer production, may influence both the chemical composition and the protein value of BG. Therefore, the aim of this study was to determine the protein value of 41 samples of BG obtained from different industrial and craft Spanish breweries. Samples were dried (40ºC) and analyzed for chemical composition. In addition, samples of each BG sample were incubated in quadruplicate with buffered sheep ruminal fluid for 12 h to determine CP rumen degradability (CPRD). The intestinal digestibility of the rumen-undegraded CP fraction (CPID) was determined using the three-step in vitro procedure, which included the incubation with pepsin solution for 1 h and pancreatin solution for 24 h. Protein value (PDIA, PDIN and PDIE) of each sample was estimated using the INRA-2007 equations and the in vitro CPRD and CPID values. For each chemical fraction analyzed, mean, minimum and maximum values, as well as the coefficient of variation (CV), were calculated. In addition, the relationship between CPRD and CPID was evaluated by linear regression analysis using the PROC REG procedure of the SAS statistical software. Content of CP ranged from 16.7 to 35.1% dry matter basis (average 25.8%), in agreement with INRA values (19.0 – 37.5%; average 26.2%). The CPRD showed high variability (coefficient of variation (CV) = 43.9%), ranging from 18.0 to 78.5% (average 36.5%), whereas CPID was less variable (10.4% CV) and ranged from 56.3 to 91.8% (average 82.2%). The average CPRD was lower than the average value of 44.0% reported by INRA for a rumen passage rate of 6%, likely due to the shorter incubation time in the rumen used in the present study (12 h; 8.3% passage rate). In contrast, the average CPID was similar to the 84.0% value reported by INRA. Average values of PDIA, PDIN and PDIE (15.3, 23.1 and 21.8% dry matter, respectively) were greater than those reported by INRA (13.7, 19.4 and 17.7% dry matter), which could be related to differences in the methodology used to estimate CPRD, as previously discussed. A strong linear negative relationship was observed between the CPID and CPRD values (CPID = 0.966 – 0.481· CPRD; R_2 = 0.83; SEM = 0.0395; $P < 0.001$), indicating that samples with higher ruminal degradability tended to have lower intestinal digestibility of the undegraded CP fraction. This highlights a trade-off between the amount of bypass protein and its intestinal digestibility, because as ruminal degradability increases, the proportion of bypass protein decreases, and its intestinal digestibility is reduced. Thus, BG constitute a valuable source of bypass protein, contributing to the supply of metabolizable protein in ruminant diets. The results indicate high variability in protein value of BG, particularly in CPRD. In practice, determining the rumen degradability of BG-CP is recommended before its inclusion in ruminant diets.

Ensiling discarded tomatoes with dried beet pulp, almond hulls or wheat bran: effects on silage quality

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A considerable proportion of total tomatoes production is lost during postharvest grading because they do not meet the high quality standards of consumers. Discarded tomatoes contain nutrients, but their high moisture content limits their use in animal feeding. Ensiling could be an effective alternative for preserving discarded tomatoes, although co-ensiling with high dry matter (DM) raw materials is necessary to improve ensiling capacity. This study evaluated the ensiling potential of mixtures of discarded tomatoes with dried beet pulp (BPT), almond hulls (AHT) or wheat bran (WBT). Each mixture was prepared to reach approximately 38-40% DM and ensiled in vacuum bags (500 g mixture/bag; 4 bags/treatment). Bags were stored at room temperature in darkness for 90 days before opening and analysis of chemical composition, pH, concentrations of volatile fatty acids, NH3-N, lactic acid, and microbial quality of silages. Data were analyzed using the software R, applying a linear model (ANOVA) with treatment as a fixed effect. Adjusted means were obtained using emmeans library, and comparisons between treatments were performed using Tukey's test. Pre-ensiling DM content was 41.6, 39.6 and 38.2% for AHT, WBT and BPT mixtures, respectively. No gas or effluent was detected in any silage, and all silages had good odor. At opening, DM content was higher ($P<0.05$) in WBT and BPT (35.4 and 34.3%, respectively) than in AHT (31.7%). Silages differed in their chemical composition (all data expressed on DM basis). Ash content was higher ($P<0.05$) in AHT (13.4%) than in WBT and BPT (7.36 and 5.81% DM, respectively). The WBT silage had the greatest protein and ether extract content (18.0 and 6.85%), whereas BPT and AHT had lower values (13.0 and 7.50% of protein; 2.43 and 3.85 % of ether extract). Neutral detergent fiber was highest ($P<0.05$) in BPT silages (47.5%), followed by AHT and WBT (44.2 and 38.5%, respectively), whereas acid detergent fiber and lignin were higher ($P<0.05$) in AHT (33.2 and 15.7%) than in WBT (14.3 and 2.56%) and BPT (25.0 and 3.90%). Fermentation characteristics also differed among mixtures. The pH was higher ($P<0.05$) in AHT (4.49) than in WBT and BPT (4.05 and 4.15, respectively), although all values were within the range considered adequate for a stable silage. DM losses were greater ($P<0.05$) for AHT (29.9%) than for WBT and BPT (13.5 and 16.8%). No propionic, butyric, isobutyric, isovaleric or valeric acids were detected in any silage. Acetic acid concentration was higher ($P<0.05$) in AHT (30.6 g/kg DM) than in WBT and BPT (25.4 and 22.5 g/kg DM, respectively). Concentration of NH_3-N was higher ($P<0.05$) in WBT (1.76 g/kg DM) than in AHT and BPT (0.49 and 0.47 g/kg DM, respectively), likely reflecting its higher protein content. No presence of pathogenic bacteria (Enterobacterias spp, Escherichia coli, Listeria monocytogenes, Salmonella spp., Staphylococcus coagulase+) were detected in any silage. Clostridium perfringens counts were low and did not differ ($P>0.05$) between treatments (3.43, 2.21 and 2.04 log colony forming units (CFU)/g for WBT, AHT and BPT, respectively). However, yeasts and molds counts were higher ($P<0.05$) in AHT (16.0 and 2.21 log CFU/g, respectively) than in WBT (12.3 and 1.98 log CFU/g, respectively) and BPT (5.65 and 2.04 log CFU/g, respectively). All treatments showed good ensilage capacity, with distinct nutritional and fermentation characteristics. Therefore, the choice of the mixture will depend on the nutritional profile desired and the availability of the raw materials.

In vitro gas production kinetics of different conventional and non-conventional feeds

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The integration of non-conventional feeds, such as agro-industrial by-products and discarded vegetables, into livestock diets is a key strategy for enhancing circularity in animal production. Including agro-industrial by-products in farm animal diets could reduce the costs of feeding and the environmental impact of their disposal. This study aimed to evaluate the in vitro gas rumen fermentation kinetics of seven feedstuffs: four conventional ingredients used in dairy sheep diets (corn, barley, soybean meal, and beet pulp) and four non-conventional feeds (discarded leek, bell pepper, and carrot). Samples (300 mg DM of each substrate) were incubated in batch cultures with diluted ruminal fluid at 39ºC during 144 h in 120 mL serum bottles (n=4). Gas production was measured at different times (3, 6, 9, 12, 17, 22, 26, 31, 36, 48, 72, 96, 120 and 144 h) for determination of gas production kinetics. Endogenous gas production was corrected by subtracting the gas volume produced by the corresponding blanks from the total gas produced in the vials. Data were fitted to a nonlinear model to estimate potential gas production (A), fractional rate (c) and lag time. Average gas production rate (AGPR) was also calculated. Gas production values were fitted to the model: Gas = A(1-e-c(t – Lag)), using the NLIN procedure of Statistical Analysis System (SAS). After 144 h, the residue was dried at 60ºC to calculate the effective degradability of the diet (DMED). Significant differences were observed among substrates for all parameters ($P<0.05$). Regarding asymptotic gas production, corn (95.87 mL/g DM), beet pulp (95.31 mL/g DM), and carrot (92.30 mL/g DM) showed the highest values, while soybean meal and leek presented the lowest (71.06 and 71.46 mL/g DM, respectively; $P<0.05$). The fractional fermentation rate and average gas production rate were significantly higher for carrot (0.066 %/h; 3.35 mL/h) and bell pepper (0.067 %/h; 3.07 mL/h) compared to conventional grains and beet pulp. Notably, corn exhibited the longest Lag time (9.23 h), whereas bell pepper showed the fastest initiation of fermentation (2.27 h). For DMED, carrot (45.85 %) and bell pepper (45.76 %) outperformed all other ingredients ($P<0.05$), including soybean meal (41.25 %) and conventional energy sources like barley (33.85 %) and corn (30.19 %). This study indicates that certain agro-industrial by-products, particularly carrots and bell peppers, appear to outperform traditional ingredients in fermentation rate and dry matter degradability. These findings would suggest that vegetable discards could serve as a sustainable substitute for costly energy sources like corn and barley, potentially enhancing the cost-effectiveness of ruminant production systems, provided further studies confirm these trends. Funding from the Spanish State Research Agency (AEI) and the European Regional Development Fund (Project PID2021-124648OB-C22) is gratefully acknowledged. N. Merino acknowledges the support of the Junta de Castilla y León for her predoctoral contract.

Assessment of gas production kinetics in dairy sheep diets including bell pepper as an ingredient replacing conventional feedstuffs

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Spain is the leading bell pepper producer in the European Union, with an annual output exceeding 1.8 million tons. This massive production is coupled with a powerful food processing industry that generates significant quantities of surplus and cull products. Within a circular economy framework, upcycling these bell pepper residues as livestock feed offers a sustainable strategy to mitigate environmental impact while reducing feeding costs for ruminants. Therefore, the aim of this study was to evaluate the inclusion of discarded bell peppers in increasing proportions in a dairy sheep diet. Four different diets (a control diet (CON), and 3 diets with different proportions of bell pepper at 8% (PEP8), 16% (PEP16) and 24% (PEP24) replacing part of the concentrate ingredients) were evaluated. Three hundred mg of each diet were incubated at 39 ºC during 144 h in 120 mL serum bottles (n=4). The gas pressure was measured at different times (3, 6, 9, 12, 17, 22, 26, 31, 36, 48, 72, 96, 120 and 144 h). At the end of the incubation, the residue was dried in an oven at 60ºC to calculate the effective degradability of the diet (DMED). Data were fitted to a nonlinear model to estimate potential gas production, fractional rate and lag time. Average gas production rate was also calculated. Gas production values were fitted to the model: $Gas = A(1-e^{-c(t-Lag)})$, using the NLIN procedure of Statistical Analysis System (SAS). The Lag time decreased linearly (P>0.05) with increasing level of pepper (4.63, 3.25, 2.20, 2.41 h for CON, PEP8, PEP16 and PEP 24, respectively). The asymptotic gas production was similar (P>0.05) for the 4 diets (72.7, 73.8, 73.8 and 73.1 mL/g for CON, PEP8, PEP16 and PEP 24, respectively). The fractional rate of gas production was different (P<0.05) between diets (0.054, 0.049, 0.046 and 0.055 %/h for CON, PEP8, PEP16 and PEP 24, respectively) being higher for CON and PEP24 diets. In relation to the average gas production rate, there was a linear increase (P<0.05) when the proportion of pepper augmented in the rations (2.07, 2.10, 2.11, 2.43 mL/h for CON, PEP8, PEP16 and PEP 24, respectively). The DMED increased linearly (P<0.05) with increasing percentage of bell pepper in the ration (32.9, 33.3, 34.6, 36.9 % for CON, PEP8, PEP16 and PEP 24, respectively). Our results demonstrate that replacing concentrate with bell pepper, particularly at the 24 % DM inclusion level, significantly accelerates the onset of fermentation and linearly increases both the average gas production rate and the effective degradability of the diet. This enhancement in ruminal efficiency suggests that bell pepper residues can potentially substitute traditional energy sources in ruminant rations. However, given the high moisture content of this by-product and the presence of bioactive compounds, further in vitro and in vivo trials are necessary to evaluate long-term effects on ruminal fermentation, animal intake, milk yield, and metabolic health. Funding from the Spanish State Research Agency (AEI) and the European Regional Development Fund (Project PID2021-124648OB-C22) is gratefully acknowledged. N. Merino acknowledges the support of the Junta de Castilla y León for her predoctoral contract.

Gas production kinetics of dairy sheep diets with increasing amounts of leek

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The region of León has many vegetable processing industries that generate a big quantity of discarded product and wastes, posing a considerable environmental challenge if not properly managed. Leek, a traditional crop in Spain, is one of the most processed vegetables in the region. Furthermore, it contains organosulfur compounds that have been reported to modulate rumen fermentation. As they are underutilized for livestock nutrition, upcycling these vegetable wastes in ruminant diets could be an option to reduce the environmental impact of agroindustry. The objective of this study was to evaluate the effect of the inclusion of different proportions of leeks in a dairy sheep diet on rumen fermentation kinetics. Four diets (a control diet (CON), and 3 diets with different proportions of leek 8% (LEE8), 16% (LEE16) and 24% (LEE24) replacing part of the concentrate ingredients) were evaluated. Three hundred mg of each diet were incubated with diluted rumen fluid at 39 ºC for 144 h in 120 mL serum bottles (n=4). The gas pressure was measured at different times (3, 6, 9, 12, 17, 22, 26, 31, 36, 48, 72, 96, 120 and 144 h). After 144 h the residue was dried in an oven at 60º C to calculate the effective degradability of the diet (DMED). Endogenous gas production was corrected by subtracting the gas volume produced by the corresponding blanks from the total gas produced in the bottles. Data were fitted to a nonlinear model to estimate potential gas production, fractional rate and lag time. Average gas production rate was also calculated. Gas production values were fitted to the model: $Gas = A(1-e^{-c(t - Lag)})$, using the NLIN procedure of Statistical Analysis System (SAS). The Lag time decreased linearly ($P>0.05$) with increasing level of leek (4.63, 3.83, 3.08, 3.11 h for CON, LEE8, LEE16 and LEE 24, respectively). The asymptotic gas production (72.7, 70.3, 72.9 and 72.1 mL/g for CON, LEE8, LEE16 and LEE 24, respectively) and the fractional rate of gas production (0.054, 0.056, 0.053 and 0.056 %/h for CON, LEE8, LEE16 and LEE 24, respectively) were similar ($P>0.05$) in the four diets. There was a linear increase ($P<0.05$) in the average gas production rate when the proportion of leek increased in the diet (2.07, 2.15, 2.22, 2.30 mL/h for CON, LEE8, LEE16 and LEE 24, respectively). Similarly, the DMED increased linearly ($P<0.05$) with increasing percentage of leek in the diet (32.9, 33.0, 34.2, 35.1 % for CON, LEE8, LEE16 and LEE 24, respectively). Our findings indicate that including up to 24% of leek in the diet, replacing conventional feeds, optimizes the onset of in vitro fermentation and nutrient utilization, supporting the development of more sustainable and cost-effective feeding systems in the region. However, further research, including in vivo trials and assessments of milk quality and animal health, is required to confirm these results and determine the long-term effects of leek's organosulfur compounds on animal performance. Funding from the Spanish State Research Agency (AEI) and the European Regional Development Fund (Project PID2021-124648OB-C22) is gratefully acknowledged. N. Merino acknowledges the support of the Junta de Castilla y León for her predoctoral contract.

Effect of replacing corn with cull carrots in the diet of dairy sheep on milk retinol and tocopherols

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There is a growing trend toward incorporating agro-industrial by-products, such as cull vegetables, into ruminant livestock diets to boost farm sustainability and support circular economy initiatives. Cull vegetables can also contain high levels of specific nutrients or bioactive compounds that may improve animal health or can be transferred to the resulting food products. However, the influence of these alternatives on the final product must be scrutinized. This research investigated how including fresh chopped cull carrots in the diet of dairy sheep affects the levels of retinol and tocopherols in the milk of dairy ewes. Two groups of ten lactating Assaf ewes on the second third of lactation were fed on two different isoproteic and isoenergetic diets (total mixed rations) for 28 days. The control group received a total mixed ration ad libitum and the other received the same ration, but corn was totally replaced by fresh cull carrots (160 g/kg DM) previously chopped in 1 cm pieces. Milk yield was recorded daily, and milk samples were taken on the last day of the experiment to analyze retinol and tocopherols. The method utilized was based on reversed-phase high performance liquid chromatography following KOH saponification. During saponification, ascorbic acid and a nitrogen purge were employed to prevent oxidation. Vitamins were recovered in hexane and redissolved in methanol. The mobile phase consisted of methanol, with detection performed via photodiode array for retinol and fluorescence for tocopherols. Data were analyzed using an analysis of variance (ANOVA) to evaluate the impact of dietary treatment on vitamin concentration. The group supplemented with cull carrots exhibited significantly higher retinol levels (exceeding 30%; $P < 0.001$). Regarding tocopherols, while alpha-tocopherol concentration was unaffected by dietary treatment, gamma-tocopherol content was approximately two-fold higher in the control group ($P < 0.001$). Notably, gamma-tocopherol concentrations remained more than ten times lower than those of alpha-tocopherol. Overall, these findings suggest a nutritional advantage for milk produced by ewes fed cull carrots. Funding from the Spanish State Research Agency (AEI) and the European Regional Development Fund (Project PID2021-124648OB-C22) is gratefully acknowledged. N. Merino acknowledges the support of the Junta de Castilla y León for her predoctoral contract.

Chemical composition, fermentative characteristics and microbial quality of wet brewers' grains ensiled with dried citrus pulp or almond hulls

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Wet brewers' grains (BG) are a valuable feed resource for ruminants due to their high protein and fiber content, but their high moisture content makes them prone to rapid spoilage and limits their practical use. Therefore, preservation techniques are needed, and ensiling is a promising option. However, the elevated moisture and low sugar content of BG pose challenges for effective silage preservation, but BG could be mixed with other raw materials for improving ensiling capacity. The aim of this study was to assess the chemical composition, fermentation end products and microbiological quality of wet BG ensiled with either dried citrus pulp (CPBG) or almond hulls (AHBG). Four silages of each treatment were prepared in vacuum bags (500 g mixture/bag) to reach about 37% dry matter (DM) in the mixture before ensiling and bags were stored for 90 days. Silages were opened and representative samples of their content were taken for measuring pH, concentrations of volatile fatty acids, NH3-N and lactic acid, and microbial quality. Data were analyzed by ANOVA with the type of silage as a fixed factor using the emmeans library of R software. The DM content pre-ensiling was 38.3 and 36.9% for AHBG and CPBG mixtures, respectively. At opening all silages exhibited good odor and coloration, with no visible mold growth or effluent formation. The CPBG silages had greater ($P<0.05$) DM losses than AHBG silages (8.70 vs. 5.32%) and lower ($P<0.05$) DM content (34.9 vs. 37.0%, respectively). The CPBG silages had lower ($P<0.05$) ash (5.04 vs. 6.77%; all results on DM basis), crude protein (16.5 vs. 17.4%), neutral detergent fiber (46.9 vs. 60.1%), acid detergent fiber (19.8 vs. 33.5%) and lignin content (4.78 vs. 14.6%), but greater water-soluble sugars content (8.43 vs. 2.05%; $P<0.001$) than AHBG silages, whereas there were no differences in ether extract levels (7.52 vs. 8.10% for CPBG and AHBG, respectively; $P=0.217$). The pH was lower ($P<0.05$) in CPBG than in AHBG (3.63 and 3.87, respectively), but all silages had pH values below the recommended value of 4.0. There were no differences between silages in the concentrations of lactic acid (49.3 and 54.7 g/kg DM for CPBG and AHBG, respectively; $P=0.100$) and acetic acid (3.28 g/kg DM for both silages; $P=0.981$), resulting in similar lactic/acetic ratios in both silages (15.1 and 16.7 for CPBG and AHBG, respectively; $P=0.207$). In contrast, NH3-N concentrations were greater in AHBG than in CPBG (6.48 vs. 0.31 g/kg DM) likely reflecting a greater CP degradation during storage in AHBG silages. Propionic acid was undetected in all silages and concentrations of butyric acid were low (average 0.058 and 0.574 g/kg DM for AHBG and CPBG silages, respectively), which are indicative of good fermentation. In agreement with these results, no presence of Clostridium perfringens, Escherichia coli, Listeria monocytogenes, Salmonella spp., Staphylococcus coagulase+ or molds were detected in any silage. The AHBG silages had greater ($P<0.001$) counts of Enterobacteriaceae spp. than the CPBG silages (3.60 vs. 2.53 log colony forming units (CFU)/g), but yeasts counts were similar (5.75 vs. 5.68 log CFU/g), being all values below the maximum recommended levels. The results suggest that both mixtures have good ensiling capacity and satisfactory microbial quality, although CPBG silages had greater DM losses during ensiling.

Variation in the composition of oil hemp (Cannabis sativa) and camelina (Camelina sativa) seeds

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Camelina and oil hemp are promising oil and protein crops, which are valued due to their drought-resistance, low input requirements and potential to substitute conventional protein feeds such as rapeseed and soy in animal diets. However, little is known about the variation of composition of camelina and oil hemp seeds, which can influence both the efficiency of oil extraction and nutritive value of residual camelina and hemp cake and meal. The objective of the study was to collect information about the variation in the nutritive value of camelina and oil hemp seeds between different years and growing locations in Finland. Seed samples (n = 59 for oil hemp and n = 15 for camelina) were collected from batches farmers provided for Finnish companies TransFarm Oy and Suomen Kasviöljyt Oy during 2021-2024. For hemp, samples were taken from batches with low (<400 kg/ha, n= 27) and high yield (>600 kg/ha, n= 32). The 1000 seed weight (SW) of camelina (only 2021 samples, n=5) and hemp (only 2021-2022 samples, n=40) were determined, and all samples were analysed for dry matter (DM), ash, crude protein (CP), total fat, ash-free neutral detergent fibre (NDF), phytate phosphorus (P), and condensed tannins (CT). In addition, glucosinolates (9-methylsulfinylnonyl, 10-methylsulfinyldecyl and 11-methylsulfinylundecyl glucosinolate) were analysed from camelina seeds. The impact of yield on composition of hemp seed was analysed with two sample t-test using TTest and Mixed procedure of SAS. In addition, the Spearman correlation coefficients of 1000 SW with the composition of hemp seeds was analysed with Corr procedure of SAS. The hypothesis was that variation in the composition of camelina and hemp seeds exists, and the yield and SW of hemp influence seed composition, as the ratio of seed surface area to volume changes when the seed size changes. The 1000 SW of camelina was on average 1.24 g (min 1.08, max 1.38 g) and that of hemp 11.8 g (min 10.6, max 13.1 g). The average DM, ash, CP, total fat, and NDF concentration of hemp seeds were 931, 55.1, 260, 332 and 334 g/kg DM, respectively, and that of camelina seeds 920, 39.6, 284, 371 and 146 g/kg DM, respectively. The variation of fat concentration in camelina (min 250, max 413 g/kg DM) and hemp seeds (min 248, max 392 g/kg DM) was high, a difference which can result in around 3 times higher oil yield between maximum and minimum fat concentration seeds if extracted to residual fat concentration of 200 g/kg DM. Phytate P concentration of camelina seeds averaged 0.500 g/100 g DM and that of hemp seeds 0.896 g/100 g DM. The concentration of condensed tannins averaged 3.81 and 1.07 g/kg defatted seed mass on camelina and hemp, respectively. Total glucosinolate concentration of camelina seeds averaged 19.4 mmol/kg DM (min 12.9, max 27.6 mmol/kg DM) and was on 67% of samples below the maximum limit of 20 mmol/kg DM allowed for 00-varieties of rapeseed in animal nutrition. 1000 SW of hemp seed tended to be positively correlated with CP concentration (P=0.081, r=0.279), indicating the potential impact of seed yield on feed quality. No other impacts of yield on or correlation of 1000 SW with hemp seed composition were observed. In conclusion, this study demonstrated a variation in seed composition of camelina and oil hemp, with relevance to both oil extraction efficiency and nutritive value of residual cake or meal. The lack of precise hemp seed yield measurements and the use of categorial yield data (<400 kg/ha vs. >600 kg/ha) provided by the farmers reduced the sensitivity of the analysis and limited our ability to detect subtle relationships between yield and seed composition.

In vitro assessment of Sicilian agro-industrial by-products and derived biochars as potential feed ingredient for animal nutrition

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In Sicily, agriculture plays a central economic role and generates substantial quantities of agro-industrial by-products from citrus fruits, olives, and pistachios food-chains, although partially reusable in livestock feeding, their high fibre content and variable composition may limit full nutritional exploitation. In this context, pyrolysis represents a sustainable valorisation strategy, enabling their transformation into biochar, an innovative, stable carbon-rich material with promising applications in animal nutrition, soil improvement, and environmental management. Recent studies suggest that biochar supplementation could be associated with improved health, nutrient utilization, reduction of greenhouse gas emissions, and microbiota modulation. This study aimed to comparatively evaluate the nutritional and functional properties of Sicilian agro-industrial by-products such as whole lemon pulp (WLP), depectinized lemon pulp (DLP), pistachio shells (PS), and olive kernel (OK) and to investigate the effects of pyrolysis on the bioactive profile of their corresponding biochars (WB, DB, PB, OB). Dried agro-industrial biomasses and their derived biochars, obtained by pyrolysis at 600°C for 60 minutes, were evaluated for bromatological composition following AOAC standard procedures. Antioxidant activity, polyphenols and flavonoids content were evaluated spectrophotometrically after solvent extraction of by-products and resulting biochars. Extracts were also tested for inhibitory activity against F18+ E. coli using a microdilution assay. Crude protein and ether extract were minimal across samples except for lemon by-products that showed higher residual content of these nutrients (CP 8.5%; EE 4.77% in DLP), PS exhibited the highest neutral detergent fiber content (92.41%), highlighting that the organic constitutes the largest component of these by-products; ashes represent the smallest component (PS: 0.35% and OK: 0.42%). OK displayed the highest antioxidant activity (1668.89 µmol Trolox eq/g ±66.69) and polyphenol content compared to other by-products ($p < 0.0001$). WLP showed the highest inhibitory activity against E. coli compared to the other samples ($p < 0.0001$), suggesting a residual presence of bioactive compounds. Biochar samples exhibited lower antioxidant activity (<10 µmol Trolox eq/g) compared with their respective raw biomasses, indicating the thermal degradation of phenolic compounds during pyrolysis. However, WB retained relatively higher antioxidant and inhibitory activity, reflecting properties of the original biomass. These findings highlight the promising potential of biochar derived from agro-industrial by-products for in vivo applications, suggesting that functional compounds are partially preserved even after the pyrolysis process. The key functional characteristic of biochar is the development of a highly porous structure during pyrolysis, which confers promising physicochemical properties and supports its potential in vivo applications, such as improving feed efficiency, modulating microbiota, promoting nutrient absorption, potentially adsorbing harmful compounds, and mitigating greenhouse gas emissions. The combination of biochar with fresh agro-industrial by-products could represent a promising strategy to valorise these residues within circular-economy frameworks, while supporting sustainable livestock nutrition and environmental mitigation.

The NUTRIFEEDS approach: Regenerative feed innovation through fermentation, insect bio-conversion and gut ecosystem modulation

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This contribution presents the Horizon Europe project NUTRIFEEDS at its initial stage, outlining the scientific rationale, key hypotheses, and methodological framework that will guide forthcoming research. It also seeks to define the concept of "regenerative nutrition", which underpins a significant part of the project. By doing so, it introduces innovative research directions, fosters collaboration, and provides early insight into integrative approaches addressing key challenges in sustainable livestock production. Sustainable livestock production requires feeding strategies that enhance nutrient efficiency, gut health, and immune resilience while reducing environmental impact and reliance on imported protein sources. The Horizon Europe project NUTRIFEEDS (2025-2029) develops regenerative feed ingredients derived from drought- and heat-tolerant plants and agri-food side streams using targeted fermentation and Black Soldier Fly (Hermetia illucens) larvae bioconversion. Raw materials are comprehensively characterized, including nutrient composition and energy value, before undergoing targeted fermentation processes designed to reduce anti-nutritional factors, improve protein availability, and enrich bioactive metabolites. Insect-mediated bioconversion further increases nutrient density and may provide functional compounds with immunomodulatory properties. These technologies aim to transform underutilized regional biomass into high-value feed components within circular production systems. Experimental feeding trials in monogastric animals and ruminants evaluate effects on nutrient digestibility, intestinal morphology, barrier integrity, gut microbial composition, fermentation patterns, systemic immune markers, behaviour, and performance. Particular emphasis is placed on microbiome-host interactions and resilience under climate-related stressors. Multi-omics analyses are combined with AI-supported modelling approaches to identify mechanistic links between feed processing technologies, microbial ecosystem shifts, and host physiological responses. Beyond biological validation, the project integrates welfare indicators and farm-level sustainability metrics to assess practical applicability. By coupling biotechnology, animal nutrition, and data-driven modelling, NUTRIFEEDS seeks to establish science-based regenerative feeding strategies that enhance gut functionality, immune competence, and productivity while reducing environmental burdens. The project contributes to the development of circular, regionally adapted feed systems aligned with the EU Farm2Fork strategy and provides a framework for improving livestock resilience through targeted modulation of the gut ecosystem.

Rapid NIRS Evaluation of Cereal–Legume Forage Mixtures from Pre-Wilting to Ensiling

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The increasing need for alternatives to conventional forage crops such as corn silage is particularly relevant in rainfed systems, where limited summer irrigation constrains livestock sustainability. Cereal-legume (CL) mixtures represent a strategic option for livestock systems, as they provide higher-protein forages, enhance on-farm feed self-sufficiency, and improve soil fertility through biological nitrogen fixation. However, the agronomic and nutritional performance of these mixtures is strongly influenced by species selection and mixture proportion, highlighting the need for accurate quality assessment. In this context, Near Infrared Spectroscopy (NIRS) offers a rapid, non-destructive, and cost-effective approach for predicting forage chemical composition and supporting feeding strategies. This study evaluated eight commercial forage mixtures differing in CL proportions: 100:0%, 50:50%, and 40:60%. The forages were sown in November 2024 at the Vallevecchia pilot farm (Veneto, Italy), harvested in May 2025, pre-wilted for 24 h, and ensiled in 14 L laboratory mini-silos for 60 days. At each stage (pre-wilted and silage), 32 samples (8 mixtures × 2 plots × 2 replicates) were analysed using official methods to determine dry matter (DM), ash, crude protein (CP), ether extract (EE), neutral detergent fibre (NDF), acid detergent fibre (ADF), net and gross acid detergent lignin (ADL), acid-insoluble ash (AIA), and starch. In addition, silage samples were evaluated for fermentation parameters, including pH, ammonia nitrogen, lactic, propionic, iso-butyric and n-butyric acids, and ethanol. Spectral data were acquired using a benchtop NIRS instrument (FOSS DS2500) in reflectance mode over the 850–2500 nm range. Separate calibration models were developed for pre-wilted and ensiled forages using modified partial least squares regression (WinISI software). Model performance was assessed by 5-fold cross-validation (cv), using the standard error (SEcv), coefficient of determination (R^2cv), and residual predictive deviation (RPDcv). Prediction performance was generally lower for pre-wilted than for silage samples. Good predictive performance was achieved only for ash and CP (R^2cv = 0.85 and 0.90; RPDcv = 2.54 and 3.10, respectively). Moderate performance was observed for gross ADL, starch, ADF, AIA, EE, and DM (R^2cv = 0.67–0.80; RPDcv = 1.72–2.26), whereas NDF and net ADL showed poor predictive performance (R^2cv < 0.66; RPDcv < 1.5). In contrast, ensiled samples exhibited markedly improved model performance. Excellent predictions (R^2cv > 0.91; RPDcv > 3.2) were obtained for DM, ash, CP, NDF, ADL, pH, and ethanol. Good predictive performance was achieved for AIA, starch, and major fermentation acids (lactic, propionic, and acetic acids), with R^2cv values between 0.82 and 0.90 and RPDcv ranging from 2.4 to 3.1. Conversely, iso- and n-butyric acids showed limited predictability (R^2cv = 0.65; RPDcv = 1.70), and EE was poorly predicted (R^2cv = 0.43; RPDcv = 1.31). Overall, NIRS proved to be a reliable tool for rapid characterization of CL mixtures, particularly at the ensiled stage. These findings highlight the potential of stage-specific calibration models to support efficient quality assessment and data-driven management of alternative forage and silage systems within sustainable livestock production. Acknowledgements: Funded by Ministry of Agriculture, Food Sovereignty and Forests of Italy, D.M.n. 0461776 del 18/09/2024 prot. n. 0544040 del 15/10/2024. Project “DI.L.CA.VE. Valori e autenticità”.

Effects of adaptation length to non-roughage finishing diets on intake, performance, and carcass traits of Bos indicus cattle

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Diets high in energy without any source of roughage have been increasingly adopted in intensive feedlot systems worldwide; however, adaptation strategies for cattle under these feeding conditions remain poorly defined, particularly regarding their effects on ruminal epithelial integrity and intake-driven performance. This study aimed to evaluate the effects of different adaptation lengths to non-roughage finishing diets on intake, performance, carcass traits, and ruminal morphometrics of Bos indicus cattle finished in a feedlot for 113 days. One hundred forty-four Nellore bulls (initial body weight [BW] 385.56 ± 7.4 kg) were blocked by BW, and randomly assigned to 1 of 36 pens (n = 4 per pen), according to the treatments: 1) Control: 14-day adaptation using a step-up protocol to a conventional finishing diet containing roughage sources; 2) 14-day restriction (R14): 14-day adaptation by quantitative restriction to a finishing diet with no roughage; and 3) 21-day restriction (R21): 21-day adaptation by quantitative restriction to a finishing diet with no roughage. Pens were treated as the experimental unit, with 12 replicates per treatment. Diets were formulated to be isoenergetic (1.29 Mcal kg^{-1} net energy for gain) and isonitrogenous. At harvest, rumens were individually scored for lesions, and a 1-cm^2 fragment of each rumen was collected from the cranial sac. The number of papillae per cm^2 of rumen wall (NOP) was determined, as well as the mean papillae area (MPA). The rumen wall absorptive surface area in cm^2 (ASA) was calculated as follows: 1 + (NOP*MPA) – (NOP*0.002). Data were analyzed using PROC MIXED of SAS, with treatment as a fixed effect and block as a random effect ($P < 0.05$). Control animals had greater final BW (566.8 vs. 519.4 and 517.8 kg; $P < 0.0001$), average daily gain (ADG; 1.56 vs. 1.18 and 1.17 kg; $P < 0.0001$), hot carcass weight (HCW; 314.4 vs. 293.2 and 284.9 kg; $P = 0.0007$), and daily dry matter intake (DMI; 9.67 vs. 7.65 and 7.46 kg; $P < 0.0001$) than cattle in R14 and R21, respectively. Rumenitis score was lower in control animals (1.89 vs. 7.16 and 7.40; $P < 0.0001$), but ruminal absorptive surface area was larger (24.97 vs. 20.0 and 19.84 cm^2; $P = 0.06$) than in cattle in R14 and R21, respectively. When all treatments were analyzed jointly, DMI was strongly associated with ADG ($r^2 = 0.739$; $P < 0.01$) and HCW ($r^2 = 0.533$; $P < 0.01$). Mixed model analysis confirmed a positive linear effect of DMI on ADG (≈ 0.15 kg increase per additional 1 kg DMI). Moreover, rumenitis score was negatively associated with DMI ($r^2 = 0.401$; $P < 0.01$). The negative association between the severity of rumen lesions and DMI implies that epithelial integrity was compromised and that voluntary DMI regulation was negatively affected. Thus, under non-roughage feeding, functional epithelial condition constrained performance by reducing DMI to cope with ruminal acidification. In conclusion, extending the adaptation period from 14 to 21 days did not improve DMI or performance on non-roughage finishing diets; thus, the original hypothesis was not supported. The DMI was the main driver of performance in this study, whereas ruminal lesion severity in cattle fed non-roughage diets limited DMI, resulting in a smaller ruminal absorptive surface area and impaired performance. Keywords: adaptation, Nellore, roughage, rumen Acknowledgments: São Paulo Research Foundation (FAPESP). Grant no. 2025/09684-7. Animal Ethics Committee – UNESP (protocol no. 003837/24).

Multi-property evaluation of late-gestation protein supplementation to improve reproductive efficiency in dry tropical beef systems

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Reproductive efficiency in beef systems depends on the ability of cows to rear a viable calf and reconceive within the same production cycle. Population-scale monitoring of dry tropical herds has demonstrated substantial variability in performance, with low pregnancy rates within four months postpartum, particularly among first-lactation cows, and median fetal and calf losses approaching 10%. Nutritional and environmental constraints have been identified as major contributors to these losses. Late gestation therefore represents a critical window during which maternal nutrition may influence neonatal survival, subsequent fertility, and overall cow–calf productivity. The objective of this study was to test whether late-gestation protein supplementation reduces total calf loss and improves postpartum reconception under commercial dry tropical conditions. Pregnant heifers and multiparous cows (n = 6,570) were enrolled across 30 commercial herd-year units from 2022 to 2024 (8 properties in 2022, 12 in 2023, 10 in 2024). Within each property-year, cows were stratified by liveweight, calving date, and lactation status and randomly allocated to routine management (Control) or late-gestation supplementation (Supplemented). Supplemented cows received a protein- and energy-rich supplement for approximately six weeks before calving, formulated to supply 300–400 g crude protein/day, 9 MJ metabolisable energy, 16 g phosphorus, and 18 g calcium for a 500 kg cow. All supplements included 1.4% Saccharomyces cerevisiae fermentation products (NaturSafe®, Diamond V, USA). Outcomes included total calf loss (from confirmed pregnancy to weaning, including missing pregnant cows), reconception rate at weaning, and pregnancy within four months postpartum (P4M). Data were analysed using generalised linear mixed models with property and treatment × property fitted as random effects. Across 30 commercial herd-year units over three years, late-gestation supplementation improved reproductive outcomes compared with Control cows. Total calf loss was lower in Supplemented cows (13.3% vs 17.2%; P = 0.04). Missing pregnant cows (assumed mortality) were numerically lower in Supplemented cows (3.4% vs 5.1%) but were not significantly affected by treatment (P = 0.12). No treatment × year interactions were detected. Supplemented cows had higher reconception rates than Controls (77.7% vs 68.1%; P = 0.02) and tended to have a greater proportion pregnant within four months postpartum (64.5% vs 53.8%; P = 0.06). Reconception differed among age classes (P = 0.03), being higher in multiparous cows (85.7%) than in two-year-old heifers (77.4%) and yearlings (49.5%), and increased with pre-supplementation weight class (P = 0.02). Late-gestation protein supplementation reduced total calf loss and improved reconception while cows were lactating under commercial dry tropical conditions. The improvement in early postpartum pregnancy is consistent with enhanced periparturient metabolic and endocrine adaptation, potentially facilitating timely progesterone decline, colostrogenesis, and earlier resumption of ovarian cyclicity. These findings indicate that nutritional systems reliant on protein-deficient forages with minimal nitrogen supplementation may impose metabolic constraints during late gestation, thereby increasing reproductive losses. Targeted nutrient supply during this period appears necessary to mitigate biologically mediated constraints on reproductive efficiency in extensive beef systems.

Multi-Trait Growth Parameter Estimation in Beef Cattle Using a Bayesian Non-Likelihood Framework

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Predicting growth parameters using both Feed Intake (FI) and Live Weight (LW) data is essential for refining feeding strategies, enhancing genetic selection, and boosting overall herd productivity. To generate such information, traditional frequentist and Bayesian models, such as the Maximum Likelihood Estimation (MaxLik) and Bayesian Likelihood (BL) methods, often rely on rigid likelihood functions that may not effectively capture biological variability or the irregular data density typical of farm-scale records. This study examines the Approximate Bayesian Computation (ABC) framework as a more robust alternative for growth parameter estimation and for consistent predictive capacity under data-degradation scenarios, using the Gompertz growth model. The framework was developed using LW records from 476 Holstein cattle bred for beef production and was refined to 453 animals with concurrent FI and LW data, collected through historical research at the Agri-Food and Biosciences Institute (AFBI), Northern Ireland, via the Northern Ireland Farm Animal Biobank (NIFAB). Data were preprocessed to eliminate outliers and individuals with insufficient longitudinal records; FI was converted to Metabolizable Energy Intake (MEI). To evaluate the efficacy of ABC, a comparative analysis was conducted against the MaxLik and BL methods. Predictive accuracy was assessed using Root Mean Square Error (RMSE), Mean Absolute Percentage Error (MAPE), and parameter coverage. Results for single-trait LW demonstrated that the ABC framework accurately recovered true growth parameters (growth rate $k \approx 0.11$month^{-1}; asymptotic weight $A \approx 1030$ kg) with a high predictive accuracy of MAPE = 3.5% and RMSE = 12 kg, representing a marginal 1.2% average deviation relative to the mature weight. By utilizing distance metrics rather than formal likelihoods, ABC achieved 99.3% empirical coverage rate for the 95% credible intervals compared to the BL approach (68.7%), offering a more reliable tool for characterizing growth trajectories. To evaluate performance under extreme scarcity, a data-degradation subset was created by reducing observations for animals with >11 records by 75%, leaving only 3–4 points per individual. In these sparse conditions, ABC yielded a lower RMSE (15 kg) than BL (16 kg), with MAPE increasing only marginally to 4.2% from 3.5% in the full dataset. Subsequently, MEI was integrated into a multi-trait ABC model, in which parameters were concurrently optimized to satisfy both traits. MEI parameters were estimated with an asymptotic intake $A2 \approx 6433$ MJ and a rate $k2 \approx 0.33$ month^{-1}. Under data degradation, the multi-trait ABC model remained stable, with MEI MAPE increasing only marginally to 24% from a 22% full-dataset baseline. While MEI RMSE values were 463 MJ and 443 MJ for sparse and full datasets, respectively, these represent a low relative error of approximately 7% of the asymptotic value, indicating high predictive stability despite the inherent volatility of intake data. Notably, ABC demonstrated superior reliability for MEI with 70.8% parameter coverage, significantly outperforming the BL approach (48.8%). These findings confirm that the ABC framework offers a robust computational method for identifying superior growth phenotypes. By maintaining high accuracy with minimal longitudinal records, this approach provides a logistically feasible solution for large-scale production settings where intensive data collection is often impractical.

Effects of adaptation protocols to non-forage high-energy diets on dry matter intake, rumen pH, and redox potential of cannulated Nellore cattle

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High-energy diets with reduced or absent forage inclusion have increased in beef cattle production systems, requiring appropriate adaptation protocols to maintain rumen health and feed efficiency. However, information regarding the metabolic responses of Nellore cattle to different adaptation protocols for non-forage high-energy diets remains limited. This study aimed to evaluate different nutritional protocols to adapt beef cattle to non-forage high-energy diets. Six cannulated Nellore cattle were enrolled in a duplicated 3 x 3 Latin square design to evaluate dry matter intake (DMI) and rumen parameters during adaptation and the entire feeding period. The experiment lasted 73 days, comprising three 21-day experimental periods with a 5-day washout interval between periods. Ruminal pH, temperature, and redox potential were continuously recorded every five minutes throughout each 21-day experimental period using a data logger. The trial was conducted, with the following treatments: 1) Control (CON): 14-day adaptation using a step-up protocol to a conventional finishing diet containing forage sources; 2) 14-day restriction (RES14): 14-day adaptation by quantitative restriction to a finishing diet with no forage; and 3) 21-day restriction (RES21): 21-day adaptation by quantitative restriction to a finishing diet with no forage. Diets were formulated on a dry matter basis using corn silage and concentrate ingredients, including non-forage fiber sources such as soybean hulls, citrus pulp, and cottonseed hulls. In the control protocol, corn silage was gradually reduced (45% to 25%) with a concomitant increase in concentrate. In the restriction protocols, cattle were fed a high-concentrate finishing diet without forage inclusion from day 1, starting at 1.2% of body weight and targeting 2.0% by the end of adaptation. Feed was offered once daily in the morning, and DMI was calculated as the difference between feed offered and refusals (DM basis). Data were analyzed using PROC MIXED (SAS, 2003), with means compared by Tukey's test. During the adaptation period, DMI, in kg/day and as % of body weight, was significantly greater in CON animals compared with those in RES14 and RES21 (10.26 vs. 7.27 and 6.42 kg, respectively; $P < 0.01$). Mean ruminal pH was reduced in cattle in RES21 compared with animals in CON and RES14 (6.11 vs. 6.52 and 6.48, respectively; $P = 0.04$), leading the rumen environment to stay longer under pH 6.2 for RES21 (922 vs. 336 and 609.55 min, respectively; $P = 0.01$). Redox potential was also affected by treatment, with a reduced value for RES14 (291 vs. 358 and 402 mV for CON and RES21, respectively; $P = 0.01$). Over the entire feeding period, DMI remained lower for cattle in RES14 and RES21 than for those in CON (7.39 and 6.42 vs. 10.31 kg, respectively; $P < 0.01$). Although mean ruminal pH was no longer different among treatments, cattle in RES14 spent more time below pH 5.2 than those in RES21 and CON (71.8 vs. 32.2 and 0, respectively; $P = 0.01$). The area under pH 6.2 also remained larger in the restricted groups (60.00 and 81.52 vs. 23.98 min; $P = 0.01$). Redox potential continued to differ among treatments, with greater values for CON and RES21 than RES14 (370.9 and 388.3 vs. 315.2 mV, respectively; $P = 0.03$). In conclusion, cattle adapting to non-forage high-concentrate diets decrease DMI as a result of ruminal acidification to avoid further variations in ruminal pH. Moreover, extending the adaptation restriction period from 14 to 21 days did not consistently improve ruminal stability.

Compositional and Nutritional Differences Among Major Forages in the Po Valley: A One-Year Analysis

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Forage in the Po Valley is critical to the Northern Italian agricultural economy, providing the foundational feed for the region's intensive dairy sector, particularly for Protected Designation of Origin (PDO) cheeses like Parmigiano Reggiano (PR) and Grana Padano (GP). Dairy production for PR cheese mainly uses forages such as hays since silage is banned to prevent Clostridium contamination and potential swelling defects in the cheese with the lengthy seasoning period. While for fresh milk and GP cheese, maize silage is the mainstay forage base production. The system is characterized by high-input and high-yield, with about 80% of Italy's dairy cattle located here. However, this system faces significant challenges due to high nutritional variability in forage, driven by climatic extremes and management decisions. Accordingly, the present study aimed to evaluate the main compositional and nutritional differences in hay and silages analyzed in the Po Valley over the past year. The data analyzed in this work derive from the analyses carried out by the Regional Association of Livestock Breeders of Lombardy (ARAL) for farmers during the last crop season (11/2024-11/2025), that included 4250 feed samples, of which about 35% were silages and hays. Nutritional composition was determined by NIRs, while dry matter was determined by oven drying. One third of the samples studied for gross analysis were represented by maize silage; other samples analysed were, in descending order: earlage, grass silage, mixed meadow hay, sorghum forage silage, wheat forage silage, and alfalfa hay. To provide a general overview, two typical forages were considered, namely maize silage and mix meadow hay. As expected the nutritional composition of both maize silage and mix meadow hay were in line with mean values reported in literature. Considering variability within forage type, the most variable nutrient in maize silage was starch with a range of value between 10% and 47%. While in the case of mix meadow hay, the most variable nutrient was protein with a range of value between 4% and 18%. In conclusion, the present study confirmed the extend of nutritional variability in the main forage crop varieties used for dairy nutrition in the Po Valley (Italy). Thus, it is essential to monitor the feed nutritional values for precise nutrition. As a future perspective, long-term nutritional studies are recommended to evaluate the impact of climatic factors on nutrient composition, potentially enabling the prediction of nutrient variability over time.

Can entire male pigs be fatter by switching the sire line from Pietrain to Duroc or increasing dietary energy?

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Until recently, processing companies were able to select carcasses with a suitable fatness for dry-curing ham process. However, when most pigs produced on commercial farms originate from lean sire lines such as Piétrain and when the proportion of entire male pigs increases, this sorting strategy is reaching its limits. The aim of the study was to determine whether selecting a fatter crossbreed or increasing dietary energy level could modify growth performance and carcass characteristics in entire male pigs. Two successive batches were studied, providing nine blocks of four pens (six to seven pigs per pen). Pens were assigned to four treatments according to a 2 × 2 factorial design combining two commercial crossbreeds (progeny of Large White × Landrace sows inseminated with Piétrain NN or Duroc semen, referred to as xPP and xDU, respectively) and two dietary energy levels. Control diets C were formulated at 9.4 MJ net energy (NE)/kg, whereas the high-energy diets (H) were formulated at 10.0 MJ NE/kg, remaining within recommended levels to avoid boar taint. Pigs were fed ad libitum and slaughtered at 96 kg carcass weight. Growth rate, feed intake, feed efficiency were characterized on the farm and carcass characteristics were determined in a commercial slaughterhouse using the Image Meater grading method (2 batches) and with a computerised tomography (CT) scanner (1 batch). No consistent crossbreed × diet interactions were detected. Over the 26 to 123 kg body weight range, the avergae daily gain averaged 1070 g/d, without any significant effect of the sire line (P=0.27) or energy level (P=0.55). The high-energy diet improved feed conversion ratio expressed in kg/kg body weight gain (H: 2.21 vs C: 2.34; P=0.005), but not in MJ NE/kg (H: 24.25 vs C: 23.57; P=0.11). It slightly increased average backfat thickness over the vertebral bodies a, b, c, and d (G4, H: 22.8 vs C: 21.2 mm, P<0.001), without affecting the minimum fat thickness on the gluteus medius muscle (G3, H: 14.2 vs C: 13.5 mm; P=0.20). Switching to xDU increased NE intake per kg (xDU: 22.4 vs xPP: 21.9 MJ/kg; P=0.006) and fat thicknesses (G4: xDU: 22.7 vs xPP: 21.3 mm and G3: xDU: 14.7 vs xPP: 12.9 mm; P<0.001). It reduced carcass leanness assessed by Image Meater (xDU: 59.9 vs xPP: 61.3%; P<0.001) or CT scan (xDU: +4.3 pct unit; P<0.001). In ascending order, G4 values were obtained for xPP pigs fed diet C, xDU pigs fed diet C, xPP pigs fed diet H, and xDU pigs fed diet H, averaging 20.4, 21.9, 22.1 and 23.4 mm, respectively. Under ad libitum conditions, carcass fatness was more responsive to crossbreeding strategy than to dietary energy level.

Impact of increased dietary phytase levels during gestating sows on offspring performance, bone mineral content and mechanical properties

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Proper skeletal development in piglets depends on sufficient maternal mineral supply during gestation. We hypothesized that increasing mineral availability in gestating sows through higher phytase supplementation would enhance placental mineral transfer and improve bone quality in newborn piglets. Therefore, this study evaluated the effects of increasing phytase levels from 300 FTU/kg to 1500 FTU/kg feed in gestating sows on offspring bone quality. A total of 60 sows (parity 0-8, equally distributed to treatments) were allocated to three dietary treatments based on phytase supplementation: Control (0 FTU group), commercial phytase level (300 FTU group), and high phytase level (1500 FTU group). Diets were provided from day 35 of gestation until farrowing. Offspring were individually weighed at birth, and mortality was recorded. One representative piglet per litter was euthanized at birth for bone sampling. Tibial mechanical properties were assessed using three-point bending, and the concentrations of Ca, P, Cu, Mn, and Zn were determined from tibial ash Performance data were analysed using a linear model. Tibial mechanical and mineral variables were using a generalized linear model with a gamma distribution and log transformation. Correlations were evaluated using Pearson's correlation coefficients. Phytase supplementation, regardless of inclusion level, had no effect on litter size (18–21 piglets per sow), total litter weight (24–27 kg), or average piglet birth weight (1.34–1.36 kg; $P > 0.05$). However, higher phytase supplementation tended to reduce litter variability, as reflected by a lower coefficient of variation, decreasing from 22–23% in the Control and 300 FTU groups to 18% in the 1500 FTU group ($P = 0.054$). In parallel, increasing phytase supplementation reduced the proportion of stillborn piglets in a dose-dependent manner, declining from nearly 17% in the 0 FTU group to about 10% at 300 FTU and 7% at 1500 FTU ($P < 0.001$). Regarding bone mineralization, tibial ash percentage did not differ among groups ($P = 0.415$), but both phosphorus and calcium concentrations increased linearly with increasing phytase levels ($P < 0.001$). Regarding the mechanical properties, although maximum compressive stress was higher in piglets from the 300 FTU group compared to Control ($P = 0.040$), no further differences were detected among treatments ($P > 0.05$). Correlation analyses revealed that Ca and P concentrations were positively associated with bone density ($r = 0.36$, $P = 0.017$; $r = 0.32$, $P = 0.033$, respectively). However, Ca was negatively correlated with bones' stiffness (Young's modulus; $r = -0.31$, $P = 0.043$) and tended to be negatively associated with proof resilience ($r = -0.27$, $P = 0.075$). Manganese concentration was negatively correlated with cortical area ($r = -0.44$, $P = 0.0027$) and second moment of inertia ($r = -0.39$, $P = 0.0087$) but positively associated with maximum compressive stress ($r = 0.35$, $P = 0.021$) and maximum tensile stress ($r = 0.31$, $P = 0.040$). In conclusion, high dosing phytase during gestation increased foetal tibial mineral deposition and reduced within-litter variability and stillbirth rate, without affecting overall sow or litter growth performance. However, enhanced mineral content did not consistently improve mechanical properties of the tibia, underscoring that skeletal resilience depends not only on mineral concentration but also on bone geometry and matrix quality.

Molecular mechanism and nutrition regulation of meat quality traits in Chinese Indigenous Pigs

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This study aims to investigate the mechanisms underlying the superior meat quality traits of Chinese local pig breeds and to establish corresponding precision nutrition regulation techniques. First, six Shaziling (S) and Yorkshire (Y) pigs at 30, 60, 90, 150, 210, and 300 d of age were selected to examine carcass traits and meat quality, in order to find the critical window for meat quality in S pigs. Our results showed that the body weight, carcass length, and loin eye area of S pigs at 150, 210, and 300 d of age were significantly lower than those of Y pigs ($P < 0.05$). S pigs at 150 and 300 d of ages had significantly lower backfat thickness than Y pigs ($P < 0.05$). Compared with Y pigs, S pigs at all 6 ages had lower lean percentage and higher fat percentage ($P < 0.05$), respectively. At 60, 90, and 150 d of age, the post-mortem pH-decline, b* value (yellowness), and drip loss of S pigs were significantly lower than those of Y pigs ($P < 0.05$). Moreover, at 150 d of age, S pigs had significantly higher a* value (redness) and intramuscular fat (IMF) content than Y pigs ($P < 0.05$). In conclusion, S pigs have a slower growth rate but better meat quality than Y pigs. The meat quality of S pigs is the best at 150 to 210 d of age. Then, to identify the top ranked metabolite enriched in metabolic pathways for the S pig meat flavor, we evaluated the composition of amino acids, fatty acids, and metabolic profiles in the longissimus thoracis muscle (LM) of S pigs aged 30, 90, 150, 210, and 300 days. Results showed that glutamate (Glu) is the top ranked metabolite enriched in metabolic pathways for the S pig meat flavor. Moreover, the muscular Glu concentration is positively correlated with total essential amino acids, flavor amino acids, and sweet amino acids of S pigs. Finally, during the critical window period for the formation of meat quality traits, we used Glu to feed S pigs in order to improve their meat quality. Our results indicated that Glu could simultaneously increase the lean percentage and IMF content and decrease the fat percentage of Shaziling pigs, and these beneficial effects may be related to increased colonic Spirochaetota abundance and acetic acid concentrations.

How deviations of requirement feed intake during mid gestation affects sows' body composition

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Feeding during early gestation typically focuses on restoring body reserves, while late gestation diets must provide increased nutrient intake to support fetal growth. During mid-gestation, nutrient requirements are assumed to be mainly for maintenance. Therefore, gestational feeding strategies, such as "high–low–high" schemes, are often used in practice. Despite their practical use, the effects of moderate deviations from recommended energy intake during mid-gestation on sow fat and muscle reserves remain unclear. This study aimed to evaluate the effects of feeding sows at 25% below, at, or 25% above estimated metabolic requirements during mid gestation (day 35-85) on backfat thickness and loin depth dynamics throughout gestation and until weaning. A total of 111 sows from seven batches (parity 1–8) were included. During early gestation (days 0–34), all sows were fed amounts calculated based on their metabolic requirements (Dourmad et al., 2017). From mid to late gestation (day 35 – 85), feeding varied by experimental group: LESS (n=38) sows were fed 25% less than calculated requirements; REQ (n=37) sows fed at requirement; and EXTRA (n=36) sows were fed at 25% above requirement. From day 85 until farrowing all sows were fed at requirement. Backfat thickness and loin depth at the P2 point were measured by B-mode ultrasound on days 0, 35, 70, 85, and 105. The number of mummies, born alive and stillborn piglets was recorded. Data were analyzed with mixed linear models including the parity and batch as random effects and for the sows body composition parameters the backfat at day 35m the sow and the interaction of time and treatment were also included (lmer, R). Backfat thickness was affected by treatment ($P = 0.005$), time ($P < 0.001$), and their interaction ($P < 0.001$). No differences were observed among treatments at the beginning of gestation. From day 85 onward, REQ and EXTRA animals exhibited greater backfat thickness compared with LESS. Backfat declined toward weaning in all groups, with no differences detected at that timepoint. Loin depth was affected by time ($P < 0.001$), but not by treatment ($P = 0.33$) or the treatment × time interaction ($P = 0.14$). Values increased from early gestation to mid-gestation and declined toward weaning across all groups. No differences were detected among nutritional treatments for litter size, number of mummies, or stillborn piglets ($P = 0.94$), litter weight at birth or at weaning ($P = 0.94$). Mid- to late-gestation feed restriction reduced backfat deposition, while the similar backfat values in REQ and EXTRA groups suggest that providing feed above requirements did not result in further fat deposition and the excess energy may have been redirected. The absence of treatment effects at weaning indicates compensatory mobilization of body reserves during late gestation and/or lactation. In contrast, loin depth was unaffected by nutritional level, indicating that moderate deviations (±25%) from requirements during mid-gestation influence fat deposition rather than muscle development. These findings suggest a higher sensitivity of body fat, than muscle reserves, to gestational feeding level and emphasize the importance of meeting nutrient requirements to maintain adequate sow body condition.

His supplementation increases muscle carnosine concentrations in weaned piglets.

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Histidine (His) is an essential amino acid in swine that participates in multiple metabolic processes beyond growth performance. It serves as a precursor of histamine, involved in digestion and inflammatory responses, and it is part of the heme group of hemoglobin, essential for oxygen transport. His is also a component of serine proteases, the pancreatic enzymes responsible for protein digestion in the small intestine. Additionally, His is part of the histidine-containing dipeptides, being carnosine (Car) one of the most important. Principally present in skeletal muscle, Car is composed of L-His and β-alanine, serving as an antioxidant, pH buffer, metal chelator and His reservoir. It has been suggested that His requirements may not be fully reflected by growth performance alone, but also by muscle Car reserves. Therefore, this study aimed to evaluate the effects of His supplementation on muscle carnosine content and growth performance of weaned piglets over time. Ten mixed-sex nursery pigs [(Landrace x Yorkshire) × Pietrain] with an initial BW of 6.18± 0.36kg and 25 days of age were allotted to two dietary treatments (n= 5) differing in the SID His:Lys ratio (32% vs 38%) for 42 days. A two-phase diet was applied with a 16% crude protein during phase 1 (0-15 days) and 18% during phase 2 (15-42 days). All pigs were individually weighed on days 0, 7, 15, 28 and 42 of the trial. During phase 1, blood samples were collected to determine free His concentrations. On days 0, 15 and 42, a sample of Longissimus dorsi (LD) of each pig was collected through needle biopsy, allowing the individual monitoring of muscle Car content throughout the study. Statistical analysis was performed using R software. Data were analyzed with mixed models as repeated measures including treatment, time and their interaction as fixed effects and individual as random. At the end of phase 1, no significant differences were detected in BW (7.67 vs. 8.26kg; P=0.262), serum free His (9.20 vs. 12.27µg/mL; P= 0.578), or muscle Car concentrations (195.5 vs.197.9mg Car/100 g muscle; P=0.956). Although BW showed numerical differences on day 42 (18.16 vs. 20.26 kg), this effect was not significant (P=0.305), likely due to the limited sample size. Finally, pigs fed His supplemented diets showed increased Car concentrations in the LD on day 42 (161 vs. 306 mg Car/100 g muscle; P<0.05). His requirements should consider muscle Car concentration due to its role as His donor, and it is concluded that piglets fed His supplemented diets showed greater Car concentration in skeletal muscle.

Acorns from intensive holm oak plantations during Montanera finishing phase: impact on the fatty acid and antioxidant profiles of Iberian pig meat

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Climate change is intensifying interannual fluctuations in natural acorn availability during the Montanera finishing phase, increasingly challenging the ability of some Iberian pigs to reach the minimum weight gain requirements established by Spanish quality standards, leading to economic losses for farmers due to animal disqualification. As a climate adaptation strategy to avoid these losses while enhancing the resilience of this traditional Iberian pig production system and preserving product quality, supplementation with acorns from intensively managed and irrigated holm oak plantations may provide a viable solution. This study aimed to evaluate the impact of this supplementation during the final Montanera phase of pure Iberian pigs on the fatty acid and antioxidant profiles. A total of 85 purebred Iberian Retinto pigs started the Montanera finishing phase under traditional conditions. After 48 days, 10 pigs with insufficient growth and 10 pigs showing adequate performance were selected. The underperforming group was supplemented with 10 kg/day of acorns from intensively managed and irrigated holm oak plantations established in 2018 using grafted plants on seedling rootstock, located in the "La Orden" Agricultural Research Center (CICYTEX) (southwestern Spain), while the control group continued under traditional Montanera conditions. Both groups completed the same 63-day finishing period consuming acorns of comparable nutritional composition. After slaughter, longissimus thoracis et lumborum (LTL) muscles were removed from the carcasses; one was subjected to a dry-curing process for 70 days under standard industrial conditions, while the other was analyzed fresh. Then, intramuscular fat was extracted for analysis of tocopherols (α- and γ-tocopherol) by HPLC and fatty acid profile by GC-FID. Results showed that, in fresh loin, acorn supplementation from intensive holm oak plantations resulted in similar α- and γ-tocopherol contents compared to traditional Montanera. No significant differences were observed in the fatty acid profile, including major fatty acids and the proportions of saturated, monounsaturated and polyunsaturated fatty acids. Likewise, in dry-cured loin, tocopherol contents and fatty acid composition were largely unaffected by supplementation, apart from a significantly lower C18:3 n-3 content in the acorn-supplemented group ($P < 0.05$). These results demonstrate that supplementation with acorns from intensive holm oak plantations preserve the distinctive fatty acid and antioxidant profiles characteristic of traditional Montanera finishing and therefore may be considered as strategy to enhance the resilience of Montanera traditional production system. Acknowledgements: This work was co-financed by the European Regional Development Fund (ERDF) through the European Commission's INTERREG VI-A Spain–Portugal (POCTEP) 2021–2027 Programme, within the project "Advanced, innovative and digital technologies for the agri-food sector EUROACE (0100_TID4AGRO_4_E)", and by the ERDF and the Regional Government of Extremadura through the project "Tradition and innovation in the production of acorn-fed Iberian pigs and the development of differentiated quality end products (IBERCAL)".

Effects of Dietary Extruded Flaxseed and Betaine on Haematological and Biochemical Parameters in Finishing Pigs

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Abstract: The use of functional feed ingredients in pig production has increased due to their potential to improve animal health and meat quality. Flaxseed (Linum usitatissimum) is a rich source of omega-3 fatty acids, particularly α-linolenic acid, and its dietary inclusion has been associated with anti-inflammatory effects, modulation of lipid metabolism and improvements in meat fatty acid profile. Betaine plays important roles in performance, carcass traits and stress resilience, potentially supporting better efficiency in pigs. Therefore, the incorporation of flaxseed and betaine in pig diets may represent a nutritional strategy to enhance both animal performance and may affect blood biochemical and haematological indicators reflecting animal health and metabolic responses. The aim of this study was to evaluate the effects of dietary extruded flaxseed supplementation, with or without betaine, on haematological and biochemical parameters in finishing pigs. The study was conducted using forty female pigs ((LW x LR) x DU) monitored during 43 days in the finishing phase, between approximately 94 and 137 kg body weight. Animals were allocated to four dietary treatments in four pens each with ten animals: control diet (T1), diet supplemented with extruded flaxseed level 1 (T2), diet supplemented with extruded flaxseed level 2 (T3), and diet supplemented with extruded flaxseed level 1 plus betaine (T4). Blood samples were collected and analysed to evaluate biochemical and haematological parameters. Data were analyzed using a variance test with feed as the variance factor and Tukey test were used for multiple comparison of means. The JMP pro 17 program was used for statistical analysis. Among the biochemical variables analysed, lactate dehydrogenase (LDH) was significantly affected by dietary treatments ($p = 0.023$). The highest values were observed in pigs receiving T4 (713 U/L), which were statistically similar to T2 (552 U/L), while T3 (482 U/L) and T1 (472 U/L) presented the lowest values. Alkaline phosphatase showed a tendency towards significance ($p = 0.054$), with higher values in pigs receiving T2 (120.60 U/L) and T4 (119.10 U/L), while animals fed the control diet (T1) presented the lowest values (96.21 U/L), like T3 (101.56 U/L). Total cholesterol also showed a tendency towards significance ($p = 0.057$). The highest values were observed in pigs receiving T1 (90.99 mg/dL) and T4 (89.56 mg/dL), whereas T2 showed the lowest values (81.22 mg/dL), followed by T3 (88.72 mg/dL). These variations may reflect metabolic effects related to lipid metabolism modulation promoted by dietary fatty acids. No significant differences were observed among treatments for the remaining haematological parameters evaluated ($p > 0.05$). These results suggest that dietary supplementation with extruded flaxseed, particularly when combined with betaine, may influence specific biochemical markers associated with metabolic activity in finishing pigs, although the general haematological profile remained stable across treatments. Keywords: Pig metabolism, haematology, flaxseed, betaine

Effects of harvest time, dry matter content and additives on fermentation characteristics of grass-clover silage

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Silage from ley crops has potential as a locally produced feed resource for pig farming, providing nutrients while decreasing reliance on more expensive protein feeds and supporting natural behaviours that enhance animal welfare. However, the nutritional quality depends on various factors, including the grass-to-legume ratio, which often varies between cuts, and the dry matter (DM) content at ensiling. This lab-scale study aimed to investigate how the grass-to-legume ratio, DM content of the ensiled herbage, and the type of additive used at ensiling influence the chemical composition of the silage intended for pig feeding. A laboratory-scale silage trial was conducted to assess the effects of harvest time, DM content and additives at ensiling, on the fermentation characteristics of grass-clover silage for pigs. A grass-legume ley, composed of perennial ryegrass (Lolium perenne) and white clover (Trifolium repens), was harvested on two occasions (1st and 2nd cuts) and ensiled with two DM contents (low, LDM; and high, HDM), and treated with no additive (CONT), a propionic-formic acid mix (ACID), or a lactic acid bacteria (Lactobacillus paracasei, L. plantarum, L. brevis) inoculant (INOC). Sixty vacuum-sealed mini-silos were prepared and analysed after three months for pH, chemical composition, ammonia-N, and fermentation products. Fermentation was influenced by the cut, DM level, additives, and their interactions. Bacterial inoculation at low DM produced silage with intense fermentation, lower pH, and higher lactic and acetic acid production. Acid treatment resulted in less intense fermentation, higher WSC content, and the lowest ammonia-N content compared to the INOC and CONT treatments. Butyric acid remained low across all treatments, except in HDM 2nd cut CONT. Overall, ACID-treated silages showed improved stability, with reduced proteolysis, making them more suitable for pig feeding. These findings suggest that combining an appropriate DM content with acid treatment of grass-legume ley could enhance silage quality, functioning as a protein source and improving feed utilisation in organic pig production scenarios.

Short-term protein-energy restriction in starter diets triggers compensatory growth, increased ghrelin and improved jejunal morphology in weanling pigs

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Nutrient supply during the immediate post-weaning period shapes both the growth trajectory and the endocrine signals that govern appetite and efficiency. In this study, we evaluated whether the nutritional restriction through a low-protein and low-energy (LP-LE) starter diet induces early growth depression but permits compensatory responses after pigs return to a balanced diet. A total of 80 [Landrace ×Yorkshire] × Duroc) weaning pigs with an average initial body weight of 6.34 ±1.15 kg were randomly assigned to 2 dietary groups with 10 replications of 4 pigs/pen for 6 weeks. Diets were fed in two phases: CON: balanced diet in phase 1 (week 0-3) and phase 2 (week 3-6); TRT: compensatory diet (LP-LE diet) in phase 1 and the balanced diet in phase 2. Growth performance [body weight (BW), average daily gain (ADG), average daily feed intake (ADFI), and gain to feed ratio (G: F)] was recorded by period (week 0-3, 3-6) and overall. Blood was collected at week 3 and 6 for appetite-related hormones, including ghrelin. Jejunal morphology (villus height, crypt depth, and villus: crypt ratio) was assessed at the end of Phase1 and 2. In addition, taste and nutrient-sensing gene expression in tongue tissue was evaluated at week 6 by qRT-PCR. All data were analysed with GLM procedure of SAS using pen as the experimental unit. $P< 0.05$ was considered as statistically significant. During the phase 1, pigs fed LP-LE diet showed significantly lower BW (13.57 vs 14.01 kg; P=0.049), ADG (347 vs 365 g/d; P=0.041) and G:F (0.675 vs 0.807; P=0.0046), but higher ADFI (517 vs 457 g/d; P=0.024) compared with CON, confirming immediate growth depression under LP-LE diet. After phase 2, ADG, ADFI, and G:F did not differ between treatments, and final BW at week 6 was similar (25.74 vs 25.96 kg; P=0.626) , indicating a recovery in growth range during the later period. Serum ghrelin at week 3 was significantly higher in TRT than CON, whereas no difference was observed at week 6. It could suggest an appetite-stimulating endocrine response to nutrient restriction, potentially facilitating subsequent compensation upon re-feeding. TRT increased villus height (459.30 vs 438.67 µm; P=0.046) and villus:crypt ratio (1.94 vs 1.76; P=0.010) while reducing crypt depth (237.04 vs 249.57 µm; P=0.049), suggesting mucosal adaptation following restriction and re-feeding. At qRT-PCR, FFAR4 of the tongue tissue showed lower expression in TRT, while no clear differences were observed in glucose transporters (SGLT1, GLUT2) or umami taste receptor genes (T1R1, T1R2, T1R3), indicating that the molecular memory of early-life restriction may be specifically linked to long-chain fatty acid signalling. Collectively, a 3-week LP–LE starter strategy transiently impairs early growth and efficiency but permits full recovery by 6-week once pigs resume a balanced diet. The concurrent rise in ghrelin and improved jejunal morphology may suggest endocrine and mucosal adaptations that may support compensatory growth. The significant down-regulation of FFAR4 serves as a molecular signature of the compensatory growth process, likely reflecting an adaptive shift in lipid-sensing sensitivity following the transition to a high-energy diet. This approach may offer flexibility for cost-sensitive starter formulations, provided that pigs are subsequently re-fed with a nutritionally adequate diet. The future study should quantify long-term carcass outcomes, integrate additional metabolic and satiety hormones and growth factors for intestinal villi to describe causal pathways. KEYWORDS: Compensatory growth, weaning pigs, ghrelin, intestinal morphology, gene expression.

Optimizing early and mid-gestation feed allowance improves litter outcomes in sows under summer heat stress

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Summer heat stress can reduces voluntary feed intake while increases maintenance energy requirements, potentially impairing energy balance in gestating sows and subsequent piglet growth. As nutrient demands change throughout gestation, the optimal timing of increas feed allowance under hot conditions remains unclear. This study investigated how increasing feed allowance during early and/or mid-gestation affects sow body weight (BW), piglet growth, inflammatory responses, stress indices, gut permeability, and fecal microbiota. Fourty crossbred gestating sows (Landrace × Yorkshire) were assigned to four treatments (n = 10 per treatment) in a 2 × 2 factorial design based on early-gestation (Control [C]: 2.3 kg/d; High [H]: 3.0 kg/d) and mid-gestation (C: 2.0 kg/d; H: 2.7 kg/d) feeding levels. Treatments were defined as H–H, H–C, C–H, and C–C. All sows received the same feed allowance (3.0 kg/d) during late gestation. Sow BW and backfat thickness were measured on d 0, 30, 75, and 110. Postweaning, serum cytokines, hair cortisol, gut permeability markers, and targeted fecal bacteria were analyzed.. Sow BW was highest in H-H and lowest in C-C at d (185.2 vs 166.4 kg; $P < 0.05$) and d 110 (199.4 vs 177.3 kg; $P < 0.01$), whereas backfat thickness and total born did not differ ($P > 0.10$). . However, stillbirths tended to be higher in H-H than C-C ($P = 0.06$). Piglet birth weight was highest in C-H ($P < 0.01$), and litter weaning weight was greatest in C-C (72.9 vs 61.4–65.9 kg; $P = 0.03$), despite individual weaning weights and weaning-to-estrus intervals being similar ($P > 0.10$). Physiological markers (cytokines, cortisol, gut permeability, andmicrobiota did not differ among treatments. In conclusion, while persistently elevated feeding (H-H) during summer increased sow BW, it tended to increase stillbirths. Conversely, stage-specific fine-tuning (such as C-H for optimal birth weight or C-C for litter weaning weight) improved reproductive outcomes without altering physiological stress markers.

Effects of Extruded Linseed and Betaine on Growth Performance of Finishing Pigs

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Dietary strategies aimed at improving pork nutritional quality have gained increasing attention in recent years. Flaxseed (Linum usitatissimum) is rich in omega-3 fatty acids, particularly α-linolenic acid, and its inclusion in pig diets has been associated with improvements in meat fatty-acid profile and potential benefits for animal performance. In addition, betaine plays important roles in performance, carcass traits and stress resilience, potentially supporting better efficiency in pigs. Therefore, the incorporation of flaxseed and betaine in pig diets may represent a nutritional strategy to enhance both animal performance and heat stress resilience. This study aimed to evaluate the effect of dietary supplementation with extruded flaxseed, with or without betain, on the growth performance of finishing pigs. Forty female pigs ((LW x LR) x DU) were monitored for 43 days during the finishing phase, from approximately 94 to 137 kg body weight. Animals were allocated to four dietary treatments in four pens each with ten animals: control diet (T1); diet supplemented with extruded flaxseed at level 1 (T2); diet supplemented with extruded flaxseed at level 2 (T3); and diet supplemented with extruded flaxseed at level 1 plus betaine (T4). Body weight and feed intake were monitored individually throughout the experimental period in automatic feeding stations (NEDAP Pig Performance Tester). Data were analyzed using a variance test with feed as the variance factor and Tukey test were used for multiple comparison of means. The JMP pro 17 program was used for statistical analysis. No significant differences were observed in initial or final body weight among treatments ($p > 0.05$). However, dietary treatments significantly affected total weight gain and average daily gain ($p = 0.001$). Pigs receiving T2 showed the highest weight gain during the trial (48.9 kg) and growth rate (1062 g/day), while T1 and T3 presented lower values (41.1 and 41.3 kg; 893 and 897 g/day, respectively). Animals fed T4 showed intermediate performance (42.3 kg; 919 g/day). Feed intake was not significantly affected by dietary treatments ($p > 0.05$). However, the animals in group T4 showed a higher consumption than the other groups (2967, 3139, 3057, 3233g/day, for T1, T2, T3, T4, respectively). Feed conversion ratio was significantly improved in pigs receiving T2 (2.96) compared with T3 (3.46) and T4 (3.52), while T1 showed intermediate values (3.33) ($p = 0.0016$). These results suggest that dietary supplementation with extruded flaxseed may influence growth performance in finishing pigs, particularly when included at appropriate levels, highlighting its potential as a nutritional strategy in pig production systems. Keywords: Finishing pigs, growth performances, flaxseed, betaine

The devil is in the details: Is longevity a good indicator of farm animal welfare?

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Animal welfare is a complex, multi-faceted concept, making farm animal assessments extremely challenging. Different actors place varying levels of importance on the different constructs of animal welfare, with these priorities often dictated by their personal values. Animal care practices are also commonly conceptualized through a sustainability lens, ideally placing equal importance on economic viability, social acceptability, and environmental impact. Not surprisingly, given the importance of economic viability, many animal welfare assessments give primary focus to whether an animal is in good health and productive. In contrast, questions about how the animal feels (e.g., absence of pain), and whether the animal is able live according to its nature (e.g., perform natural behaviours that are thought to be important to it, such as grazing) receive much less attention in on-farm animal welfare assessments. Within the discussions surrounding farm animal welfare and sustainability, the issue of longevity has been introduced as a possible indicator of animal welfare. Longevity in humans is defined by the Merriam-Webster dictionary as pertaining to the length of life, and as such, has been used as a key indicator when assessing quality of life and well-being. This indicator is arguably well positioned to assess human well-being given the associations between long life, good health, and happiness. However, the adoption of longevity as an indicator of welfare in farm animals is less straightforward. The length of a farm animal's life is more likely to depend on key economic drivers, rather than the animal's quality of life. In this presentation we will discuss why the term 'longevity' must not be treated as a one-dimensional concept. Rather, increasing the length of a farm animal's life presents potential advantages and disadvantages when it comes to animal welfare and sustainability more broadly. For example, within the dairy cattle literature, longevity has often been argued to be a key indicator of welfare, yet involuntary culling – the killing of animals that are sick or injured – can lead to improved animal welfare by reducing suffering and shortening length of life. In contrast, given that dairy cattle are a significant contributor to the meat supply, we argue that the slaughter of a healthy younger animal would likely be more accepted by society than keeping the animal alive longer when risks to welfare are high, such as during long-duration transportation. Moreover, there is a growing body of evidence that the public does not expect that farm animals live a long life but rather that their life, however short, has meaning and purpose, including through their contribution to the food supply.

Genetics meets management: Explaining the gap between genetic and phenotypic trends of longevity in dairy cattle

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Longevity is one of the key traits in dairy cattle breeding, as it directly influences herd structure and replacement rates and thereby contributes to overall production efficiency. In addition to its economic relevance, longevity has gained attention in societal and political discussions, where it is often perceived as an indicator of animal welfare. Between 2010 and 2024, considerable genetic progress in longevity was achieved, with an average annual increase of 24.4 days. In contrast, the corresponding phenotypic trend exhibited a substantially lower rate of increase, amounting to only 9.6 additional days per year in the same period.To investigate the discrepancy between genetic improvement and phenotypic trends, we performed comprehensive analyses using data from German Holstein cattle, encompassing disposal years 2010 to 2025. Several hypotheses were tested, considering external influences and management practices as potential explanatory factors for the observed trends.Our findings provide strong evidence that management practices constitute the principal determinants of phenotypic longevity. For instance, the milk price crisis in 2015/2016 had a strong negative impact on phenotypic longevity, as economic pressure prompted management decisions such as herd downsizing or prioritizing culling over treatment. Further stratified analysis by herd size revealed that larger herds generally exhibited shorter longevity compared to herds with fewer than 200 cows, indicating that structural and management-related factors linked to herd scale may substantially influence longevity of the cows.Another key factor identified was replacement and insemination management. The use of sexed semen and beef semen in dairy herds alters the availability of purebred female calves suitable for herd replacement. A greater supply of replacement animals increases selection intensity and may lead to earlier culling, thereby reducing phenotypic longevity independently of the animals' health status. This indicates that cows are frequently unable to fully express their genetic potential, as they are culled prematurely due to management-related decisions. Such practices contribute significantly to the observed discrepancy between the genetic and phenotypic trend.Overall, the results clearly demonstrate that various management factors play a dominant role in phenotypic longevity, while animal welfare and health seem to have a comparatively minor impact. Most culling decisions are driven by management and economics rather than health-related issues. Consequently, longevity should not be interpreted as a reliable proxy indicator of animal welfare.

Investigations on an optimal longevity of dairy cows in practical farms in the North-East of Germany

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Aim of the analyses was to investigate when a cow should be culled because of her age. The question was whether cows that are too old slow down breeding progress to such an extent that it has negative consequences for the dairy farm's economy. Therefore, we used data of breeding organizations RinderAllianz and Rinderproduktion Berlin-Brandenburg with testherd information from 2024. Data included 68,251 German Holstein cows with 22,088 cullings. The influence of milk yield (305-ECM), lifetime performance, lifetime efficiency, health (every first veterinary treatment per diagnosis), and somatic cell count on contribution margin was analysed using a mixed linear model using SAS software (version 9.4, SAS Institute Inc.). Contribution margin was calculated including cost of heifers, male calves, feed, labour, veterinary treatments per diagnosis, labour efforts per diagnosis, inhibitor milk, variable rearing costs as well as milk revenues and revenues of culled cows and male calves. Results show, that a cow should be milked up to 7th lactation, from point of view of milk yield (breeding value included). There is no threshold responding lifetime efficiency. Health of dairy cows decreased with every lactation. Mastitis increased from first to 4th lactation by a factor of 2.4. Claw and leg disorders increased by a factor of 2.1 from first to 4th lactation. But very old cows (9th and more lactations) seemed to have a better claw health. Disorders of metabolism were 4 times higher in the 4th lactation compared to first lactating cows. Somatic cell count exceeded the limit of 400,000 cells/ml from the 4th lactation onwards. Genetically, young cows are the best. But cows that have grown old were once the best too. For somatic cell count cows up to 6th lactation had a breeding value above 100 points. The relative breeding value of milk yield fell below 100 points from the fourth lactation onwards (with an annual depreciation of 130 kg of milk). The relative breeding value of longevity was above 100 points until the fifth lactation (with an annual depreciation of 2.4 points). Cullings of first lactating cows decreased within last 8 years from 24 % of all culled cows within herds to only 14 % in 2024. Contribution margins per cow and lactation increased up to 8th and more lactations. No limit lactation number could be determined. More data with significantly older cows are necessary. With regard to the contribution margin no limit of age of dairy cows was calculated in this analyses. More cows with 8 and more lactations are necessary for further investigations. An optimal age composition of dairy herds should contain 20 % first lactating cows, 18 % of cows in second lactation, 16 % in 3rd, 14 % in 4th and a minimum of 5 % in 8th and more lactations. Older cows have a higher risk of illness. Therefore, prevention, hygiene, and early disease detection are essential. Another way to reduce critical phases of dairy cows' life is an extended lactation.

Mortality and lifetime milk yields of Finnish dairy cows during 2011 - 2020

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Mortality among dairy cow herds includes animals that have died on farm, either unassisted or by euthanasia. High mortality indicates impaired animal welfare and represents wasted resources: 1) dead cows exit the food chain, 2) deaths are often preceded with work and other used resources, and 3) mortality may shorten herds´ productive lifespan and lead to raising more replacement heifers. Through those, mortality potentially influences carbon footprint of milk production. Mortality is affected by decisions of dairy farmers who adjust feeding, management and housing of their herds based on their own values and knowledge, expectations set by dairies and legislation, and fluctuating market prices of milk and meat. Because of these, sizes of dairy herds are increasing and housing changes from tie stall to free stall barns in many European countries. In some countries, including Finland, farmers also shift from conventional to automatic milking systems (AMS) and from other breeds to Holstein (HOL) cows. Along these changes, mortality of cows has increased. Despite that, only a handful of studies have analysed long-term trends in mortality, and none of them separated unassisted and euthanised deaths. Our aim was to gain more knowledge with which dairy cow mortality could be decreased in the future. We explored annual herd-level recordings of 3 179 Finnish dairy herds that were actively producing milk during 2011 to 2020, using descriptive statistics and generalized mixed-effects regression models. Of the studied herd-years, 83 % were with both Ayrshire and HOL cows in herd, and average milk yield of 9 064 kg in 2011 and 9 879 kg in 2020. In Finland, herds with less than 40 cows are mostly housed in tie stall barn, and herds with 40 or more cows in free stall barns. In our data, 41 % of 40 – 99 cow herds and 68 % of herds with 100 or more cows were milked with AMS. We observed a U-shaped trend in overall mortality (OM), which was due to changes in proportion of euthanised cows (EUT, between 4 to 5 %). Unassisted deaths (DIED) remained stable (at 2 %). As a result of changes in OM and a linear decrease in culling % (from 33 to 30 %), proportion of mortality of total culling (MTC) first decreased (from 19 % in 2011 to 18 % in 2013) and then increased, being 24 % in 2020. OM, EUT, DIED and MTC percentages were all at lower levels in herds with less than 40 cows and at higher level in herds with 100 or more cows, compared herds with 40 to 99 cows. However, within those three groups, no clear associations were observed between OM and number of cows in a herd. In contrast, EUT % increased linearly over time along the increase in HOL cows % in the herd, in all those herd size groups. Bigger herds had higher proportions of HOL cows (55 % in 100 or more cow herds; 46 % in 40 – 99 cow herds; 37 % in less than 40 cow herds). Average age at culling (AGE) increased by 8 months and lifetime milk yield (LMY) by 4 800 kg (from 25 129 to 29 893 kg) between 2011 and 2020. The association between AGE and HOL % was U-shaped, but LMY increased with proportion of HOL cows in herd. Our results suggest that observed differences in mortality between herds of various sizes are more probably reflections of many factors (like selection of breeds) than actual effects of number of cows.

Longitudinal evidence that genomic functional longevity reflects biological resilience in Holstein Friesian cows

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Extending functional longevity in high-producing dairy cows is essential for improving economic efficiency, environmental sustainability, and animal welfare in modern production systems. This longitudinal experimental study validated the biological expression of Genomic Functional Longevity Breeding Values (GFLBV) in a stratified cohort of Holstein Friesian F0 cows managed under uniform conditions for over a decade. Pregnant heifers were genomically classified into High-GFLBV (n = 18) and Low-GFLBV (n = 18) groups while being matched for Genomic Milk Production Breeding Values (GMPBV), thereby isolating survival-related genetic effects from production potential. Lifetime phenotypes, including milk yield, milk composition, reproductive performance, survival time, and detailed veterinary diagnoses, were recorded and analyzed using estimation-based statistics and Kaplan–Meier survival modeling. Milk production trajectories and energy-corrected yield were comparable between groups across parities, confirming successful decoupling of production intensity from longevity. Despite equivalent yield, Low-GFLBV cows exhibited consistently higher weighted disease burden beginning in lactation 1, initially characterized by severe mastitis clustering and transition-related disorders, progressing to multimorbidity dominated by infectious and mechanical claw lesions in lactation 2, and consolidating into a lameness–fertility constraint by lactation 3. Attrition accelerated markedly in Low-GFLBV cows from lactations 2–5, with complete cohort extinction by lactation 6, whereas High-GFLBV cows persisted into lactations 7–9. Survival analysis demonstrated sustained separation of hazard curves, confirming extended median survival in High-GFLBV cows. These findings provide rare long-term experimental evidence that genomic functional longevity captures intrinsic immunometabolic robustness and structural resilience independent of milk yield, supporting its integration into breeding programs targeting sustainable lifetime performance.

Sow longevity: phenotypic time trends, the effect of litter size, and economic values

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We analyzed data of 352,904 PIC-Camborough® parent sows housed in 322 commercial farms across the world. From sow birthyear 2000 to 2024, litter size (total number born, TNB) steadily increased each year by 0.16 piglets per litter. We performed a continuous-time survival analysis on these data, stratified by sow birthyear, adjusting for a random farm effect, with the average lifetime TNB per sow as a curvilinear covariate. From 2000 to 2023, the general sow survival rates decreased steadily with tentative signs of a turnaround since 2022. However, relative to the average TNB of 12.7 piglets per litter, the estimated hazard ratios (i.e. the probabilities of not surviving) for sows with lower or higher TNB decreased over time (i.e. their survival improved over time). We also performed a discrete-time survival analysis on these data, stratified by sow birthyear and adjusting for a random farm effect. This produced estimates of the conditional survival probabilities (CSP) per parity number per sow birthyear; these CSP estimates were used to derive the marginal economic values (MEVs) of TNB and of the sow survival rate up to parity 4 (S(p4)). Due to the upward time trend in TNB and the downward time trend in S(p4), the MEVs of these traits have gradually grown close together, so that their contributions to a profit-oriented breeding goal are becoming very similar. These data on Camborough sows show a similar overall reduction of sow longevity over time as is reported in the literature, but they hold no evidence for an important effect of increasing TNB levels in this process. In these data the optimum TNB level with respect to sow longevity has increased from just over 11 in sow birthyear 2000 to almost 17 in 2023.

Long-life sows: how breeding performance tailors longevity in breeding farms

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Elevated mortality and high removal rate of breeding sows is becoming a growing problem in the swine industry due to concerns about the management, physical health, and wellbeing of commercial sows. Thence, the objective of this study was to assess the survival curves of breeding sows and evaluate how breeding performance records impact their longevity in commercial conditions. Data from 557 Spanish breeding farms were extracted through the BDporc platform (www.bdporc.irta.es/en) retaining a total of 2,693,711 historical cycle records from 565,621 sows removed between 2022 and 2024 (both included). A COX model was developed at the sow level to estimate sows' probability to survive to the next breeding cycle based on lifetime, breeding parameters (age at first mating, average first-mating-to-conception interval, abortion incidence, average number of piglets born alive and weaned, and average weaning-to-breeding interval), season, and year as categorical factors and farm as a random effect. The overall survival probability of commercial sows decreased linearly from cycle one to five (50 % probability) and exponentially thereafter to less than 5% in cycle nine. Indeed, 16 % of sows did not reach their second breeding cycle. Besides the high variability across farms, breeding problems (infertility, anestrous, and abortions) and prolificity throughout their lifetime were common drivers of early sow removal and mortality. Sows with long weaning-to-breeding intervals (> 20 days) had 2.75 greater odds of removal than sows bred in less than a week after weaning. Also, sows that took more than three weeks to get pregnant had 88 % more chances of being removed than those that conceived in less than a week. To a lesser extent, the hazard ratio was significantly higher (1.16-1.17) for sows that have had a miscarriage throughout their lifetime than those that never had an abortion. In terms of prolificity, sows that farrowed and weaned more than 10 piglets per parity had the highest survival likelihood throughout their breeding career whilst those nurturing more than 20 piglets per litter had 84 % higher odds of removal before the next breeding cycle. Sow survival was also significantly influenced by age at first mating, season, and year. Altogether, these results highlight that breeding problems and low prolificity are priming factors of early removal and mortality in commercial sows. On the other side, farrowing and nurturing large litters (> 20 piglets per parity) can undermine the life expectancy of hyperprolific sows in commercial breeding barns. These results support the close link between sow breeding performance and longevity. Practically, efforts to prevent breeding problems may help reduce high early removal rate and mortality in breeding farms, and limiting litter size to 20 piglets per sow during lactation may also support the longevity of multiparous sows.

A multi model, multi imputation framework for predicting sow culling risk across parities

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Sow culling and mortality are a health and economic challenge for pig producers. Traditional parametric lineal models to study and identify risk factors for sow culling are challenging due to limited sample sizes, high individual variability, biology shifts across parities and collinearity among recorded parameters. Hence, we studied the use of statistical learning models as a tool to identify risks factors for culling. We hypothesised that adding data from biomarkers obtained from saliva, colostrum and milk would enhance predictive accuracy of parity-specific culling risk models beyond what is achievable with traditional analysis using routinely collected production traits alone. We used data from 120 sows monitored longitudinally over up to five consecutive parities at our pig research unit. A two-phase modelling workflow was developed to evaluate our hypothesis. In the first phase, models incorporated only routine production traits, including body condition measures, feed intake traits, reproductive outputs and Δ values for body weight, body condition score and backfat, calculated as the difference between weaning and farrowing. All predictors were standardised, and missing data were imputed using ten-fold multivariate imputation by chained equations. Three modelling approaches: full logistic regression, stepwise AIC regression, and elastic net regression with cross-validation were fitted across all imputed datasets for each parity to characterise collinearity, establish a parsimonious baseline, and identify stable variable selection behaviour. In phase two, biomarker panels from saliva, colostrum and milk were added to the same base variables used in phase one. Biomarkers were categorised by sampling stage: peak of lactation (day 10 of lactation) and the day before weaning (day 27). Stability selection was applied to quantify the consistency with which each biomarker was selected across imputations and subsampling, producing both selection frequencies and empirical p-values. Model performance was compared using Area Under the Curve (AUC) between models created from phase one data and models created from phase two data. Routine traits alone produced moderate predictive ability with distinct parity-specific effects and high collinearity. Biomarkers combined with routine traits showed statistically significant improvements during early parities. The saliva biomarker panel produced the largest improvement in parity one, improving the AUC from 0.62 to 0.76 for a ΔAUC ~0.14, with extremely strong support from Fisher combined p values ~10^{-21}. The saliva biomarkers with the highest stability across parities were C-reactive protein and amylase at weaning, and advanced oxidation protein products and cortisol at peak lactation; all exceeding our threshold for stability (> 0.30). The colostrum biomarker panel showed similar predictive improvement (ΔAUC ~0.13). Milk biomarkers showed more modest improvements (ΔAUC ~0.076). By parity four, improvements were minimal across all biomarker panels, likely reflecting a physiological ceiling effect in which routine data already captures much of the decline associated with late parity culling risk. In conclusion, this two-phase, multi-model and multi-imputation framework provides a robust and biologically interpretable method for predicting sow culling risk. Elastic net regression emerges as the most reliable and stable modelling approach. Biomarker integration, particularly saliva biomarkers measured at weaning and peak lactation, substantially enhances prediction in early parities. These findings highlight the potential of biomarker-assisted risk assessment for precision management within commercial sow farms.

Extensification is not one-dimensional: contrasting welfare and meat quality responses to space, enrichment and roughage in fattening pigs

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Extensification strategies are increasingly promoted to improve pig welfare and align production with societal expectations and evolving legislation. However, the consequences of implementing extensification measures in otherwise intensive systems remain poorly understood across welfare and production outcomes. This work, conducted within the mEATquality project (European Union grant no. 101000344), assessed how three individual extensification measures (increased space allowance, point-source enrichment, and daily roughage provision) affect welfare, growth, and meat quality in finishing pigs under intensive conditions. Across two production batches, pigs were exposed to increased space allowances (1.4 m²/pig, n=108 or 2.1 m²/pig, n=72), point-source enrichment (ropes, paper bags, and wood logs, n=214), or roughage (2-5 kg per pen per day, n=215), and compared with a conventional control (0.7 m²/pig, n=215). Welfare outcomes included damaging behaviours, pen fouling, gastric ulcerations, feeder access, and night-time resting behaviour. Increasing space allowance reduced tail biting and ear biting (up to 88% fewer biting events in the treatment with the most space), and pen fouling (up to 90% fewer events, $p<0.05$). Increased space was also associated with improved access to the feeder (up to 75% fewer observations of blocked feeder headspaces in the week prior to slaughter) compared with the control ($p<0.01$). Increased space also influenced resting at night, with pigs given more space having a higher probability of lying on the solid floor and with limited body contact compared with the control ($p<0.01$). However, the treatment with the most space was also associated with a higher prevalence of gastric ulcerations (approximately 50% of the pigs, $p<0.05$), possibly due to a greater intake of highly concentrated feed, as reflected in a greater growth rate. In contrast, roughage provision reduced gastric ulcerations by 40% but was associated with increased ear biting and pen fouling, possibly due to increased competition when roughage was provided directly in a pile on the floor. Similarly, point enrichment did not prevent tail biting outbreaks and even exacerbated problems once an outbreak had developed, possibly due to increased competition around enrichment points. Overall, responses differed across welfare indicators, demonstrating that extensification measures do not uniformly improve welfare, as unintended negative effects occurred in certain contexts. Extensification factors had limited effects on meat quality traits. However, pigs showed approximately 5% increased growth and carcass weight in the treatment with the greatest space allowance compared with control and roughage treatments ($p<0.05$). Experimental batch significantly affected several meat quality and growth parameters ($p<0.01$), indicating that environmental and management variation had a stronger influence than extensification factors and/or that the tested strategies were too moderate to affect meat quality. Overall, the findings show that extensification measures influence welfare and production components in distinct and sometimes contrasting ways, highlighting trade-offs rather than uniform improvements. These results suggest that resilience in pigs is unlikely to be achieved through single housing or management modifications implemented in isolation; instead, extensification strategies should integrate improvements across multiple aspects, including housing, feeding, enrichment, and management, to support animal resilience.

Straw in, straw out: Do quantity and location of straw affect pig health in welfare label farms?

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Welfare label farms are an emerging trend in Austria, which account for 5% of the pork production with a rising tendency. The most common label farm is characterized by a reduced stocking density (1.4m2/pig at 110kg), the use of straw, different floor types, functional areas and access to an outdoor area. All of these measures are known to improve pig welfare as they support natural behavior (e.g., foraging/lying) and can alleviate behavioral disorders (e.g., tail biting). Nevertheless, there is less evidence wether welfare systems have health benefits. Therefore, the aim of this study was to investigate the effect of straw location and quantity on health-related slaughter data. Over a period of one year, data of 23 welfare label farms in Austria were gathered. The farms were divided into two groups: 1) retrofit farms with a mostly fully slatted indoor area (feeding and elimination) but high straw provision (>50 g/day/animal) in the outdoor lying area (HighStraw_OUT; n=10) and 2) usually newly built farms with low straw provision indoors in the lying area (<50 g/day/animal) and a partially slatted outdoor area without straw for feeding and elimination (LowStraw_IN; n=13). Slaughter data in terms of pathological changes of lung (pneumonia), pleura (pleurisy) and heart (pericarditis) as well as adhesion of the pluck, abscesses and arthritis were collected by official veterinarians for each farm. A Wilcoxon rank-sum test was carried out for all six health parameters to test for differences between the farm types. The median prevalence of pneumonia was 25.6%, which is in line with other studies. HighStraw_OUT animals had significantly more pneumonia than LowStraw_IN pigs (median: 39% vs. 22.7%; p=0.021). The prevalence of all the other respiratory health related parameters did not differ between farm types and was generally low (median pleurisy: 10.3%; pericarditis: 2.2%; adhesion of the pluck: 1%). Arthritis which can be associated with an inadequate lying area (amongst other reasons) did not differ between farm types but was numerically lower in HighStraw_OUT (median: 0.2% vs. 0.4%; p=0.943). The prevalence of abscesses possibly caused by tail-biting or other systemic infections did also not differ between HighStraw_OUT and LowStraw_IN (median: 1.2% vs. 1.1%; p=0.724). It has to be underlined that these findings are preliminary and have to be interpreted with caution as they do not account for other abattoir-, farm- and pen-specific factors. However, they were collected across a whole year and for in total 35.577 pigs which provides a reasonable sample size. The prevalence of respiratory problems was higher compared to other studies. The higher prevalence of pneumonia in HighStraw_Out pigs could be explained by no clear separation of functional areas in this farm type, since pigs may use the slatted flooring indoors as well as the deep straw outdoors as elimination area. Hence, higher ammonia concentrations in the lying area could cause lung problems, even though the lying area is in the outdoor run with less dust exposure compared to the LowStraw_IN systems. Here, pigs lie indoors and do barely eliminate in that area. The lack of difference concerning arthritis and abcesses may be explained by the low prevalence in both systems and their multifactorial nature. These results suggest that there is a need to improve respiratory health across both welfare label systems. However, especially for HighStraw_OUT systems further investigations are necessary to identify influencing factors (e.g., ammonia emissions, ventilation, manure management). Based on this, improvement measures can be implemented on existing farms and should be considered when designing new ones.

Effects of farrowing in pens or crates on sows and piglets

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Worldwide, farrowing in crates has become the standard practice. This achieves work efficiency but is also expected to protect the piglets, but it also raises animal welfare issues. In Germany, like in other European countries, the legislator has imposed 2021 significant requirements on pig farmers for new buildings, as well as for existing buildings after a transition period. Farrowing must take place in pens in the future with a minimum pen area of 6.5 m2, and fixing the sow in farrowing crates is only permitted for a maximum of 5 days around the expected time of birth. However, breeding and housing systems have developed and adapted in parallel over decades. Therefore, in addition to the many questions regarding structural and organizational implementation, the question arises for this study how free farrowing affects sows and their piglets around farrowing. In experimental approaches, we monitored a total of 115 farrowings at the pig facility of the Research Institute for Farm Animal Biology (FBN). Sows of two breeds, German Landrace (n=44) and German Saddleback (n=37), farrowed either once or twice in a farrowing crate (1 m²) or in a farrowing pen (6.5 m²). The birth processes were documented and repeated blood samples were taken from sows (n=46, from 2 days (d) before to 4d after farrowing) and piglets (n=144, on d0, d1, d4, d20, d29). The influences of birth induction and piglet weight were also considered. It was found that essential hormones for birth (oxytocin, prostaglandin F metabolites), metabolism (glucose, NEFA, BHB), or stress (cortisol, adrenaline, noradrenaline) in sows before, during, and after birth were not significantly influenced by the type of housing over the entire period. Only the stress hormone adrenaline was significantly elevated in sows in crates on particular days following birth. The interfarrowing intervals also did not differ between the housing systems (median of 18.4 min in crates and 16.8 min in pens). It was noticeable that 46% of German Landrace animals experienced at least one birth interruption of 60 minutes or more, while this occurred in only 12% of Saddleback animals. However, the occurrence of dystocia was not influenced by the housing systems. When assessed for vitality using the APGAR score modified according to Randal (1971; 0 to 8 points), the piglets showed comparably good vitality immediately after birth, with an average of 7.5 out of a possible 8 points in both housing systems. Although the piglets reached the udder significantly earlier in sows kept in crates, with a median of 13 minutes compared to 18 minutes in the pens, the first colostrum intake at the teat occurred at a median of 19.5 and 22 minutes in the crates and pens, respectively, which was comparable. Successful colostrum intake could be demonstrated by the total protein in the piglets' blood. Piglets in both housing systems showed a similar and good colostrum supply on the first day of life, with 5.3 ± 0.1 and 5.1 ± 0.1 g/dL total protein in the plasma. Until weaning, we observed comparable weight development in both housing systems in this study. Overall, we observed piglet losses of 14.1% of piglets born in crates and 15.4% in pens for German Landrace sows, and 16.0% and 15.3% in these housing systems for German Saddleback pigs, with losses mainly occurring up to the third day of life. According to our research, switching to specially designed, animal friendly farrowing pens should result in a physiological birth process and no negative effects on piglets or sows are to be expected. However, different pen designs must be taken into account and, in individual cases (e.g., aggressive sows), the option of briefly restraining the sow should be used to reduce early piglet losses.

Balancing productivity and welfare to improve resilience in modern pig farming systems

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The term resilience refers to the capacity of a system to respond to both extreme disturbances, and chronic stress. A resilient system should be able to absorb such impacts, then recover and adapt. Although resilience is often considered in the context of how a system can respond, preparation for such events is an essential component of resilience. During the last century both pig farming systems, and pigs themselves, have become extremely efficient in their operational and performance capacity when all is 'going well'. Breeding strategies have produced sows that can deliver ever increasing numbers of piglets, and both advances in genetics and nutritional science have meant that the growth rate and efficiency of pigs has had a similar upward trajectory. These improvements are generally considered a success story for the industry, yet the infrastructure and management systems that have co-evolved to maximise these gains in performance have resulted in pigs approaching the limits of their capacity for resilience. We are entering an era where the resilience of both pigs, and the farming systems in which they live, are likely to be further tested; potential disruptions and stressors are likely to arise from a multitude of sources, including the threat of emerging disease (e.g. ASF), environmental challenges (e.g. extreme weather events), and consumer and political pressure to raise welfare standards. Although it cannot be measured directly, there are many ways to estimate resilience in farm animals, from immunological measurements, to measuring stress response or performance. One of the easiest and most specific methods to estimate resilience in pigs is to consider their propensity to perform tail biting behaviour. Although a common problem in pig production, it is considered an abnormal behaviour because it is rarely performed even by modern commercial strains of pigs when managed in an environment similar to that within which the species evolved. Widely accepted to be a symptom of the experience of chronic or acute stress, it emerged as a systemic issue during the early 20th century when pig production intensified. To control it, the docking of pigs tails became a routine practice. This enabled pigs to be managed in a way that maximised economic efficiency; recommendations and regulations regarding stocking densities, feeder space allowances etc. were subsequently developed using docked pigs as the model. However, the removal of the tail did not reduce stress levels per se, it simply reduced the likelihood of this behavioural response occurring. Moreover, tail biting continues to be a significant production problem, indicating that pigs are often unable to successfully cope with stressors. There are two approaches which could be employed to resolve this mis-match between the pig and their environment. The first is to improve the resilience of the pigs themselves; this is of increasing importance as an industry breeding goal. Measures of tail damage could aid in estimating the ability of the animals to cope with stressors and 'bounce back' to optimal production levels. The second is to redesign pig rearing systems so that they better meet the behavioural needs of pigs, reduce the need to dock, yet remain economically viable. This approach aligns with meeting the requirements of the EU pig directive (Council Directive 2008/120/EC), and could also address consumer and citizen concerns regarding the welfare of farmed animals. Reducing the pigs' allometric load in this way will increase their resilience, improve the robustness of farming systems, and potentially provide opportunities for diversification into novel rearing strategies that could add value to the final product.

Castration alternatives in European pig production: Welfare outcomes, technical feasibility and adoption trends

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Surgical castration of male piglets remains a widespread practice in Europe to prevent boar taint, despite well-documented welfare concerns associated with acute pain responses, stress-related behaviours and tissue inflammation. The EFSA Scientific Opinion on pig welfare recommends either systematic use of effective anaesthesia and analgesia or transition toward entire male production or immunocastration. However, adoption of these alternatives varies considerably across Member States due to differences in slaughter weight, market requirements, regulatory frameworks and consumer acceptance. Within the Horizon Europe project WelFarmers, a structured review was conducted combining peer-reviewed literature, grey literature, national regulations and updated country-level data (2009–2024). The objective was to evaluate technical constraints, welfare implications and implementation trends of alternatives to painful surgical castration. Data indicate substantial divergence across Europe. Between 2016 and 2024, surgical castration decreased from approximately 80% to 49% in France, with entire male production rising to 48%. In Portugal, castration declined from 12.5% to <5%, and more than 50% of males are currently reared entire. Conversely, surgical castration remains above 90% in several Member States, illustrating persistent structural heterogeneity. From a technical perspective, intratesticular and subscrotal administration of local anaesthetics (e.g., lidocaine or mepivacaine at 0.5 mL per testis, 5–10 min prior to surgery) significantly reduces behavioural pain indicators compared to surgery without anaesthesia, although complete pain suppression is not achieved. Analgesics (e.g., meloxicam, flunixin) mitigate post-operative inflammatory responses but show variable effects on acute pain behaviours. Immunocastration (two-dose anti-GnRH vaccination protocol, second injection 4–6 weeks before slaughter) effectively reduces boar taint compounds and modifies behaviour after the second dose but increases labour input and requires strict compliance and operator safety measures. Entire male production eliminates surgical pain but is associated with increased mounting and aggression, particularly at slaughter weights around 110 kg, requiring stable grouping, sex separation and adapted feeding strategies. Overall, measurable progress toward castration avoidance is occurring in several EU countries; however, technical feasibility remains strongly conditioned by production systems and market structures. Harmonised pain mitigation protocols and context-adapted adoption of alternatives represent realistic pathways for improving welfare without compromising productivity. AcknowledgementsThis work was supported by the European Union's Horizon Europe Research and Innovation Programme under Grant Agreement No. 101134117 (WelFarmers).

Duration of muscle excitations during inert gas stunning of slaughter pigs: Do they influence meat quality?

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Stunning pigs with inert gases is considered advantageous from an animal welfare perspective compared to stunning with high concentrations of carbon dioxide (CO2). However, it has not yet been implemented in commercial slaughterhouses as for instance, concerns regarding meat quality persist. These include lower pH45 values, which is suggested to be caused by longer or stronger muscle excitations during stunning. In this context, blood lactate can serve as an indicator of acute muscle activity and metabolic state. We therefore hypothesized that longer excitation durations (ED) would be associated with elevated blood lactate levels and reduced pH45. The present study aimed to investigate these relationships in the context of inert gas stunning. Data were derived from the second experiment of the project "Testing inert gases in order to establish replacements for high concentration CO2 stunning". A total of 900 crossbred slaughter pigs (slaughter weight M = 98 kg, SD = 8 kg) were stunned on 14 slaughter days using argon (Ar), a nitrogen-argon mixture (N2-Ar), or at least 90% CO2 (control). The experiment was conducted with a commercially used dip-lift system retrofitted with a new gassing technology achieving a residual O2 concentration of < 1%. Pigs were stunned in pairs and the process was video recorded. ED was evaluated by two observers using randomized video sequences. Blood samples collected during exsanguination, were analyzed for lactate concentration. Meat quality traits (pH & temperature) were assessed in the M. longissimus thoracis et lumborum (LTL) and M. semimembranosus (SM) at 45 min and 24 h p.m.. The final dataset comprised 244 (Ar), 244 (N2-Ar), and 246 (CO2) pigs with all traits recorded. Inert gas stunning resulted in no significant reduction in the SM pH45, while for LTL a reduction ($P < 0.001$) was found between the inert gases and CO2. Even though this reduction was significant, the numerical differences did not affect other meat quality parameters as no pale, soft, and exudative meat was detected. However, blood lactate concentrations and excitation durations showed significantly higher values in pigs stunned with inert gases, whereas CO2-stunned pigs exhibited lower lactate levels and shorter excitation durations ($P < 0.05$). To find indicators of a relationship between ED, blood lactate concentration, and pH45, correlation analyses were conducted. ED was only weakly correlated with pH45 values in the SM across inert gases ($r > -0.2$, $P < 0.05$) and was statistically significant solely in the N2-Ar group for the LTL. Similarly, only a weak correlation was found between ED and lactate for N2-Ar ($r < 0.2$, $P < 0.05$). In contrast, blood lactate concentrations were significantly negatively correlated with pH45 values for all gases. CO2 and Ar exhibited moderate negative correlations ($r < -0.2$, $P < 0.05$), whereas a weak correlation for N2-Ar was found ($r > -0.2$, $P < 0.05$). Taken together, these findings suggest that excitation duration is only a coarse measure for muscle pH variation. Instead, lactate production appears to have a stronger correlative relationship with muscle pH, indicating that strength of muscle activity needs to be taken into account. To better understand the physiological mechanisms linking inert gas stunning to metabolic responses and p.m. muscle physiology further investigations are needed. Understanding the mechanisms behind the pH45 reduction in valuable muscles during inert gas stunning could ease the future implementation of this method in commercial slaughterhouses.

Longitudinal study: Swine inflammation and necrosis syndrome in suckling piglets is associated with tail length and integrity in slaughter pigs of two organic farms in the Netherlands

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The purpose of the study was to evaluate whether clinical signs of the Swine Inflammation and Necrosis Syndrome (SINS) in piglets from organic farming systems could serve as a prognostic indicator for later tail health. Therefore piglets from two organic farms in the Netherlands were longitudinally studied from birth until slaughter. Additionally, the association between hematological parameters in weaners and the clinical outcome at different ages was tested. A total of 361 piglets with long tails from two closed organic farms in the Netherlands were digitally identified and clinically assessed for signs of inflammation and necrosis (SINS) as suckling piglets and again as weaners. The tail base, tail tip, teats, ears, coronary bands, and heels were examined. Tail integrity was assessed again during the finishing period, and tail length and integrity were evaluated at the slaughterhouse. Individual carcass weight, backfat thickness, and muscle thickness were recorded. Complete datasets were available for 200 pigs. Blood samples from 35 pigs were collected at weaning and analyzed hematologically. Signs of SINS were detected in 80% of suckling piglets and 24% of weaners. Small tail lesions without obvious biting were found in 10.3% of finishers. All finishers with tail wounds, had shown signs of SINS as suckling piglets ($P = 0.011$). Among examined slaughter pigs, 76% exhibited a tail length >30 cm, while 23% had tail lengths between 23 and 30 cm. A significant effect of the SINS score in suckling piglets on the prevalence of tails >30 cm at slaughter was detected ($P < 0.001$). The probability of exhibiting a tail >30 cm decreased with increasing SINS scores recorded during the suckling phase: 99.9%, 89.0%, 68.7%, 83.6%, 58.8%, and 0% for SINS score groups 0 (lowest scores) to 5 (highest scores), respectively. There was an association between tail length and tail lesions at slaughter. In pigs with tails >30 cm, 90.6% showed no abnormalities, compared with 67.9% in pigs with tails <30 cm ($P < 0.001$). No associations were detected between the SINS score of suckling piglets and the occurrence of healed wounds or local size deviations of the tail at slaughter. However, the SINS score was significantly associated with the absence of any tail abnormalities. No correlations were found between SINS scores and carcass parameters. Hematological indicators of white and red blood cells at weaning were significantly associated with SINS signs observed during the suckling phase. With increasing SINS scores, the following significant changes were observed: an increased monocyte percentage, a decreased thrombocyte count, a decreased erythrocyte count, a decreased lymphocyte count, a decreased hematocrit and a decreased red cell distribution width. Clinical signs of inflammation and necrosis in suckling piglets were associated with tail length and integrity at slaughter, confirming the suitability of SINS as a prognostic indicator for later tail damage. This indicator can be used as an animal-based measure to improve pig welfare, as reflected by tail integrity. Hematological parameters in weaners were correlated with SINS signs in suckling piglets, supporting the inflammatory nature of the syndrome. The associated hematological parameters may serve as supportive diagnostic tools for the prevention and evaluation of SINS in pigs.

Status quo of pre-weaning mortality in organic farms in Germany

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Pre-weaning mortality presents a serious economic and ethical problem. The expectations for organic farms are therefore particularly high. This study aims to describe the current situation on farms and subsequently identify causes and possible solutions by exploring the housing system on farm, fertility data and using microbiological diagnostics. Therefore, a comprehensive questionnaire was developed covering base data, farm organisation and management, genetics and, if applicable, vaccinations programs of sows and piglets, castration procedures, causes of mortality, documentation, structural conditions, feeding of sows and piglets, management during the peripartal time and piglet rearing. From December 2024 to September 2025, 62 farms with five to 960 sows from Germany have been included. On average, they had space for 125 ± 20 sows, but only 112 ± 20 sows were kept. This may be due to economic factors, but also to uncertainties regarding animal diseases. Both, modern genetics and old breeds were used. Most farms (85.5%) housed pigs in stables with outdoor access around parturition and during the suckling period. Size and design of the pens varied greatly. Only 4.8% of farms had outdoor housing, and 9.7% of farms had a mixed form of indoor and outdoor housing. Regardless of housing ($P > 0.05$), farms reported pre-weaning mortalities between 5.9% and 36.0%, on average 16.4 ± 0.8%. However, pre-weaning mortality could not be precisely determined for each farm, so farms with unverified data were excluded from this analysis. Therefore, to minimise pre-weaning mortality in some farms, it is necessary to fully document and record the extent and causes of pre-weaning mortality in order to address the problem. Because newborn piglets have to deal with a lot of challenges and the parturition process itself also affects the piglets' survival later on, parturition monitoring is important. However, the intensity varies between farms. Some farms use video monitoring, while most check the sows occasionally during the day. All farms stated that they provide obstetric assistance themselves, when necessary, on 30% of farms, a veterinarian is also called if needed. Different management measures could improve the piglet survival. For example, 35.5% of the farms places the piglets are placed at the udder to ensure colostrum intake, or cross-forstering is performed at 93.6% of the farms. The level of implementation of these and other measures varies between farms. While some provide intensive care for piglets in the first few days of life, others only carry out absolutely necessary procedures. In summary, it should be noted that mortality rates range from 5.9% to 36.0%. The conditions in organic piglet production are very heterogeneous, which requires individual solutions to reduce pre-weaning mortality. It is essential to record extent and causes of pre-weaning mortality in order to systematically remedy problems. In a second part of the project, swab and faecal samples will be taken from a total of 20 farms, to determine early microbiota colonisation and establish possible links to piglet health and pre-weaning mortality The project is funded by the Federal Ministry of Food, Agriculture and Rural Affairs (BMLEH) and managed by the Federal Office for Agriculture and Food (BLE) as part of the Federal Organic Farming Programme (acronym: ProFerkel).

Histopathological characterization of ear damage caused by ear biting and aggression in recently weaned pigs

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Ear lesions (i.e. bite marks, scratches, ear necrosis) are common in pigs in intensive production systems. Aggressive behaviour is considered the primary cause of ear scratches, which are hypothesised to contribute to ear necrosis by providing an avenue for bacteria to enter the ear. Ear-directed oral manipulation (e.g. biting, suckling, nibbling, chewing) is thought to result in bite marks, which are often not as macroscopically visible as scratches. Nevertheless, they could also predispose the pig to ear necrosis if the underlying tissue is damaged. Ear necrosis is considered the most severe type of ear lesion, as it can result in complete degradation of the pinna. This study recorded behaviour in pre-weaned piglets, then compared "bite marks" arising from ear-directed oral manipulative behaviour, and "scratches" caused by aggressive behaviour, using histopathological methods. Sows and piglets in conventional farrowing crates were managed according to routine farm practices. Twenty litters (n=293 piglets) were observed from birth once/wk for 4 wks. All occurrences of ear-directed oral manipulation were recorded during five 3-min observation sessions (n=15 min/litter/wk). Pigs were mixed at weaning (28d of age) into pens of 10 pigs. On d4 post-weaning 40 pigs were euthanised. Both ear pinnae were collected, and biopsies of the ear were taken when bite marks or scratches were observed. In total, 20 biopsies from 10 pigs (2 samples/pig: 1 bite mark and 1 scratch on the same ear) were processed and graded for changes in 17 histopathological parameters on a scale of 0-3 (0=absent, 1=mild, 2=moderate, 3=severe). For each parameter, diagnostic agreement between paired lesions on the same individual pig (bite vs scratch) was determined using Cohen's weighted kappa (K) and exact percent agreement (proportion of pigs where both damage types received the same score). Differences between damage types (across all pigs) were tested using the Wilcoxon rank-sum test. All litters performed ear-directed oral manipulation pre-weaning and most (12/20 litters) even in the first week of life (mean±SD; wk 1: 0.08±0.090, wk 2: 0.33±0.217, wk 3: 0.39±0.328, wk 4: 0.47±0.275 manipulations per pig/15-min/litter). Dermal fibrosis, vasculitis, and vascular hypertrophy were absent in all samples. Mild orthokeratotic hyperkeratosis, mild to moderate epidermal hyperplasia, mild perivascular inflammation, mild to moderate oedema, and neutrophil presence were present in all samples. Within the same pig, 8 parameters had a high (>50%) percent agreement, while the K value was poor for all parameters (range: -0.18 to 0.41, all $P>0.05$) across bite marks and scratches. When damage types were grouped across all pigs, scratches had higher scores than bite marks for epidermal necrosis, clefting, pustules, exocytosis, serocellular crusting, and oedema (all $P<0.05$), while scores were similar for the remaining 8 parameters ($P>0.05$). Although ear-directed oral manipulation in piglets caused minimal macroscopic damage, these findings indicate similarity with scratches, which are much more visible, in the type of tissue damage at histopathological level. This study also confirmed that pigs perform ear-directed oral manipulation as early as 1 week of age and continue to perform the behaviour throughout the suckling period. Confirmation that early life ear-directed oral manipulative behaviour results in histopathological lesions suggests a possible influence of this early life behaviour on post-weaning development of ear necrosis and warrants further investigation.

Preweaning mortality in laboratory mouse breeding: an animal science and veterinary perspective on a 3R challenge

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High preweaning mortality is a major welfare and economic problem in laboratory mouse breeding. This talk will present a summary of more than a decade of research into this often-overlooked problem. Despite greater environmental protection, preweaning mortality in mice is substantially higher than for litter-bearing mammals reared for food production animals. In the first large multi-site study under practice conditions, we showed that mortality varies greatly between facilities and is substantially larger than reference data. In data from 35,000 litters of C57BL/6 mice we found 39% preweaning mortality in one facility and 14% in the other, whereas reference data is 8%. This study also identified litter overlap, advanced dam age, and very small or very large litters as major risk factors for pre-weaning death (Morello et al 2020 doi.org/10.1371/journal.pone.0236290). Different inspection routines and record keeping may partly explain the disparity in results. Comparison of data from high-resolution behavioural monitoring from video recordings with standard pup counting methods has demonstrated that routine daily cage checks substantially underestimate perinatal deaths by underestimating the number of pups born Analysis of this video footage also indicates that unlike the popular belief that females kill pups, infanticide is rare and when pups are lost it is mainly through cannibalism of pups that were already dead (Brajon et al 2021 https://doi.org/10.3390/ani11082327). Parallel post-mortem analysis using a standardized neonatal necropsy protocol provide information on causes of death (Capas-Peneda et al 2020 doi.org/10.1177/0023677220983374). Preliminary results show that among pups that were born alive but die shortly after birth, failure to obtain milk (stomachs without milk spots) is common, sometimes with air in the stomach suggesting a failed attempt to suckle (Capas-Peneda et al 2023 10.1016/j.jcpa.2024.03.079). Appropriate temperature and nest quality can mitigate some of the risks associated with the social configuration in the cage (Morello et al 2024 http://dx.doi.org/10.1038/s42003-024-06654-z). Our most recent results also suggest that managing the social configuration by moving periparturient females may be a successful strategy to avoid risky litter overlap and improve pup survival. From the perspective of our team of animal scientists, biologists and laboratory animal veterinarians, it is surprising that so little attention has been paid to the problem of early pup mortality. The impact on the logistics of research animal facilities is substantial, especially taking into consideration that often the entire litter is lost. Mortality happens mainly within the first three days after birth. The practice in many animal facilities not to inspect breeding females until several days after parturition likely means that many animal facilities are unaware of the problem. Also, whereas many animal facilities have digital systems for animal management, they typically lack human resouces capacity to extract data from these systems to inform management practice.

Animal welfare definitions, frameworks, and assessment tools: Advancing the measurement and laying the foundation for improved animal welfare through a three-step approach

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Animal welfare assessment tools are being developed and existing tools are being modified to make them applicable to multiple species living under different management and housing conditions. These assessments should produce reliable, consistent and reproducible results that can be used to guide animal welfare-related activities. Here, we discuss the key steps for developing, validating and applying animal welfare assessment tools. These steps are: 1) agreeing on a definition of animal welfare; 2) formulating and agreeing on a framework for evaluating animal welfare; and 3) developing and validating animal welfare assessment tools based on the adopted welfare definition and evaluation framework(s). However, the development of many of the assessment tools currently in use has not systematically followed this three-step approach, and the theoretical basis on which an assessment tool is developed remains unclear. Both theoretical or conceptual frameworks—which structure the research and indicate which aspects are prioritized—and ethical frameworks—which clarify the underlying moral stance—should be taken into account. Increasing transparency and clarity regarding the underlying definitions and framework will make it more likely that the resulting welfare assessment tools will give similar weight to the aspects considered relevant to animal welfare. The proposed three-step approach should lead to consistent welfare assessment results, facilitate the positioning of these tools within ongoing welfare discussions and clarify which welfare concepts have been applied when developing the assessment tool.

Rethinking animal welfare through the microbiota: early maternal microbial contact builds immune resilience in commercial broilers

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Modern commercial broiler production relies on centralized incubation and rearing, which significantly improves production efficiency. However, this practice separates chicks from hens, depriving them of their natural early life environment. This separation disrupts the vertical transmission of microbiota, resulting in a delayed and incomplete establishment of the early life gut microbiota, which consequently becomes dominated by environmental microbiota. Research indicates that early gut colonizers are not passive residents but key regulators of host physiological functions. Increasing evidence highlights their role in improving behavioral welfare by reducing fearfulness and mitigating feather pecking. Beyond these behavioral effects, the early gut microbiota also plays critical roles in intestinal barrier function, immune maturation, and nutrient metabolism. Given the critical impact of the early gut microbiota on host health, the concept of animal welfare should extend beyond physical comfort to include natural microbial colonization. Consequently, early microbiota intervention in commercial settings provides a feasible welfare strategy aligned with the 3Rs principles. Rather than relying on medical interventions after disease onset, this proactive approach utilizes early microbial transfer to establish a stable health foundation from the beginning. To evaluate this concept, we performed an experimental model that exposed newly hatched chicks to maternal microbiota to mimic the natural way of microbiota acquisition. We investigated whether this early contact could modulate gut microbiota and metabolite production to enhance resilience against a lipopolysaccharide (LPS) induced immune challenge. Our findings demonstrated that maternal microbial contact significantly increased cecal microbial diversity and altered community structures. These changes were accompanied by enhanced production of cecal volatile fatty acids and modulated serum tryptophan metabolism. Notably, maternal microbial contact significantly interacted with the LPS challenge, effectively modulating LPS induced alterations in both microbial and metabolite profiles. Independent of the LPS challenge, maternal microbial contact upregulated genes related to intestinal barrier integrity and antioxidant defense. Conversely, the LPS challenge independently increased the expression of proinflammatory cytokines and impaired mucosal barrier function. In conclusion, early maternal microbial contact effectively modulated the gut microbiota and metabolite production, representing a potential strategy to improve immune resilience in broilers. This demonstrates that integrating natural biological mechanisms into commercial husbandry is not merely a health intervention, but also a potential welfare improvement.

Ruminant specific multi enzymatic in vitro model accurately predicts in vivo feedstuffs degradability and digestibility to phase out of fistulated reference trials

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Precise evaluation of forages and raw materials nutritional content is essential for designing cost-effective and sustainable ruminant diets. Organic matter digestibility (OMD) and ruminal dry matter (DMD) and nitrogen (ND) degradability parameters are key determinants of ruminant diet's nutritional value and zootechnical performance. Historically, reference values for DMD, ND and OMD have been established using fistulated animals through in sacco and gas test (HFT) methods respectively. However, these approaches raise ethical questions.In vitro enzymatic models represent a credible alternative to in vivo assays, although existing methodologies are outdated and fail to characterize nutrient degradability kinetics up to 48 hours. Therefore, the goal of this study was to develop an in vitro enzymatic model capable of accurately predicting in vivo ruminal degradability kinetics and OMD for a wide range of feedstuffs. In vitro model parameters (enzyme-to-substrate ratio, stirring speed, pH and enzymatic activities) were established based on ruminal fluids measurements and supplemented with data from scientific literature. Ruminal fluid samples were collected from six fistulated cows in compliance with ethical requirements (APAFIS #21558). A series of experimental designs was then conducted to identify key conditions, notably enzymes dosages and optimal pH conditions. The resulting enzyme cocktail comprises more than 20 distinct enzymatic activities (e.g., cellulolytic, hemicellulolytic, xylanolytic). Once parameters set, the in vitro model was applied to 29 feedstuffs chosen to cover the high diversity of ruminant diets (forages, oilseed meals, cereals and by-products) and selected from our in vivo trial repository. Residues were analyzed after 2, 4, 7, 24 and 48 hours of incubation to generate digestion kinetic curves. As an initial validation, the in vitro model's performance was assessed by 1) measuring repeatability (3 replicates) and reproducibility (2 operators, 2 days each) on 16 different feedstuffs with average CV of 1.1% and 2.3%, respectively; 2) comparing in vitro vs in vivo outcomes using linear regression model. The coefficient of correlation (R_2) was calculated on the 4 hours incubation time point of the kinetic curve for DMD parameter with high performance ($R_2 = 0.84$). Building on this proof of concept, further development was performed on the 29 feedstuffs and 30 different samples of grass silage to develop a specific prediction model. Four machine learning algorithms (Elastic Net, PLS, Ridge and multiple linear regressions) were trained on 25 samples to predict in vivo DMD and ND kinetics from in vitro curves and silage's chemical composition. Model performance on predictive accuracy was evaluated by the root mean square error of prediction (RMSEP) on a held-out test set of 5 samples. On the 29 feedstuffs, predicted DMD and ND kinetics were strongly correlated with in vivo curves ($R^2 = 0.97$ and 0.94 respectively) and the global hierarchy among and within the feedstuffs was preserved. For grass silage, the best-performing machine learning model achieved RMSEP values of 4.2% for DMD and 3.3% for ND. The most accurate prediction of in vivo OMD yielded an RMSEP of 1.4 % for OMD. Although ingredient specific models remain to be developed for other feedstuffs to expand the range of feeds evaluated with this new method, these results demonstrate the strong potential of coupling advanced in vitro enzymatic methods with mathematical modeling for accurately predicting in vivo nutritional values in ruminants to replace fistulated cows and address public concern on animal testing.

Accurate prediction of apparent metabolizable energy of feedstuffs using a poultry-specific in vitro digestion model

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Accurate evaluation of feedstuffs' energy value is essential for cost-efficient and sustainable poultry feed formulation, particularly to facilitate the inclusion of alternative raw materials such as by-products. Apparent metabolizable energy is usually determined in vivo, which is costly, time-consuming, and relies on animal experimentation. In contrast, in vitro digestion models are widely used in human nutrition but remain underdeveloped for poultry, despite their strong potential to support the 3Rs principle, and enable rapid feedstuffs screening. The goal of this study was to develop predictive models of in vivo apparent metabolizable energy for a wide range of feedstuffs, based on in vitro digestible energy (IVDE), measured with our poultry-specific semi-dynamic digestion model. In vitro model parameters (enzyme-to-substrate ratios, pH and enzymatic activities) were established using measurements on gastrointestinal (GI) contents, further supplemented with data from scientific literature. GI samples were collected from roosters (n=10) and broiler chicks (n=36) exclusively to parameterize the assay and in compliance with ethical approval requirements (APAFIS#21033-2019061206096709v2). Routine IVDE determinations are subsequently performed using an in-house developed cocktail of commercial enzymes. A total of 175 raw materials representing the variability of eight major poultry feedstuffs were selected: wheat (n=52), corn (n=36), barley (n=8), soybean meal (n=22), sunflower meal (n=25), rapeseed meal (n=18), wheat DDGS (n=7) and faba bean (n=7). Each raw material was evaluated as a paired in vivo–in vitro sample and analyzed for chemical composition (dry matter (DM), ash, crude protein, ether extract, crude fiber). In vivo apparent metabolizable energy corrected to zero nitrogen balance (AMEn) was measured in roosters according to the European reference method based on total collection (Bourdillon et al., 1990) and ranged from 1650 to 3900 kcal/kg DM. IVDE was measured in triplicate. Data were analyzed using linear models (R software). Model performance was evaluated on a held-out test set, and root mean square error of prediction (RMSEP) was calculated using cross-validation techniques, to assess predictive accuracy on independent raw materials. IVDE repeatability ranges from 1.1% to 2.5% and reproducibility from 1.4% to 3.5%. These values are equivalent, or even slightly better than those measured in vivo. A feedstuff-specific linear model combining IVDE and chemical composition predicts AMEn with a coefficient of determination (R^2) of 0.988 and a RMSEP of 71 kcal/kg DM. By suppressing the feedstuff effect, a global model achieves a R^2 of 0.976 and a RMSEP of 101 kcal/kg DM. This model could be used for new raw materials. These results demonstrate the strong potential of this poultry-specific in vitro digestion model as a reliable tool for predicting in vivo energy value, supporting more efficient and sustainable feed formulation. Beyond AMEn evaluation, this model offers promising perspectives for the assessment of feed additives such as NSP-degrading enzymes.

Validation of an AI-Based Method for Corneal Endothelial Cell Viability Assessment in an ex vivo Porcine Model

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The success and safety of corneal transplantation rely on accurate quality assessment of donor corneal tissues. Endothelial cell density (ECD) is currently the primary parameter used to evaluate corneal suitability; however, it does not provide a comprehensive assessment of tissue health. The evaluation of endothelial cell viability offers complementary and clinically relevant information, enabling a more reliable selection process. In line with the principles of Replacement, Reduction, and Refinement (3Rs), this study employed an ex vivo porcine corneal model using slaughterhouse by-products, thereby avoiding the use of live animals. As only tissues derived from the food chain were used, no ethical approval was required; all procedures complied with European Eye Bank Association (EEBA) guidelines. A U-Net convolutional neural network (CNN) was developed to automatically classify corneal endothelial cells according to viability status, based on Trypan Blue staining in microphotographs. Images were acquired using a Nikon Eclipse Ts2R inverted microscope at 200X magnification. The dataset comprised 230 images from twenty porcine corneas for model training using Masked Image Modeling (MIM) software, and 25 images from an independent set for validation, with a strict cornea-level split to prevent data leakage. Manual annotations were performed in a blinded manner by a trained operator using ImageJ-Fiji and were used as the Gold Standard for model evaluation. The model was trained to identify three classes: viable cells, non-viable cells, and undefined or out-of-focus regions. During evaluation, undefined areas were excluded from quantitative analysis by masking them from the denominators. The CNN successfully distinguished the three cellular classes, achieving an overall counting accuracy of 91%, with class-specific accuracies of 93% for viable cells and 70% for non-viable cells. In addition to its accuracy, the model markedly reduced processing time from approximately eight hours to a few minutes. Beyond improving efficiency, the automated approach enhances reproducibility and minimizes operator-dependent variability, contributing to methodological refinement. Overall, this study validates an AI-based, image-driven method for endothelial viability assessment in an ex vivo porcine model. By integrating computational analysis with ex vivo methodologies, this approach supports a 3Rs-oriented framework, refines experimental procedures, and reduces animal use in translational ophthalmic research. Further studies involving larger datasets and different imaging systems are planned to improve model robustness and broaden its applicability.

Left-side dominance in laying hens: an investigation of chick lateralization following a 10-week exercise regime

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Effective, coordinated wing-flapping flight is critical for safe movement and collision avoidance in skeletally vulnerable laying hens living in commercial aviary systems. Variation in contralateral neural control has been documented in domestic pullets, including right-foot preferences (indicative of left-hemisphere dominance of the hindlimbs) and a tendency to turn left (right-hemisphere dominance over the wings). These asymmetries may reflect broader motor lateralization, potentially influencing flight biomechanics and muscle development. Although early-life exercise can promote musculoskeletal growth, its effects on forelimb motor lateralization remain unclear. Thus, this study investigates whether controlled flapping descent (CFD) training induces asymmetric flight muscle development (as evidence of lateralization and motor modularity) and improves keel bone traits. A total of 168, day-old female chicks (84 white, 84 brown), were allocated to 12 pens (14/pen), randomized and stratified by strain. Within each pen, 7 chicks were assigned to CFD training and 7 to control (CON); the experimental unit was the individual bird. CFD training occurred within each pen and involved catching all chicks and placing CFD chicks individually onto a 1.2 m platform to descend onto a 20-cm-high soft-landing mat. Training occurred 5 days/week from 1–10 weeks of age, starting at 1 descent/day and increasing by 1 every two days. At 11 weeks, bodyweight, flight muscle thickness and keel bone traits (ossification %, total area) were measured by a blinded observer via ultrasound and radiography. Effects of treatment and strain were analyzed using a generalized linear mixed model in SAS, including their interaction as fixed effects, pen as a random effect, and bodyweight as a covariate. This study was approved by the University of Guelph Animal Care Committee (AUP # 5173). The left pectoralis (PEC) of CFD pullets was significantly thicker than CON pullets at 11 weeks of age ($p=0.0005$). Additionally, CFD chicks exhibited a greater left PEC:SUPR (supracoracoideus) ratio ($p = 0.0005$) and increased left PEC thickness relative to body weight ($p = 0.0002$) compared with CON chicks. CFD predominantly stimulated the PEC, responsible for the downstroke, while the SUPR, which drives the upstroke, appeared comparatively less involved. Observed hypertrophy of the left PEC may indicate coordinated motor lateralization, whereby established right-leg dominance is associated with enhanced activation of the left wing. White-feathered pullets showed greater flight-supporting development than brown pullets: thicker left and right PEC and SUPR muscles, larger keel areas, and a greater proportion of keel ossification (all $p<0.0001$). These findings indicate lateralized development of the flight muscles, particularly hypertrophy of the left PEC in pullets, aligning with previously reported right-foot dominance. This further supports the theory of contralateral hemisphere dominance of the hind- and fore-limbs. In aviary housing systems, recognizing such lateralization and motor modularity could guide decisions on perch configuration, ramp alignment, and flight pathway design to better accommodate natural movement patterns.

Implementation of flight-maneuver training in commercial rearing: No extra energy costs

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Controlled flapping descent (CFD) is a type of locomotion characterized by wing flapping during descent from an elevated surface. Training laying-hen pullets to complete CFDs is proposed to strengthen flight muscles, maneuverability and potentially reduce keel bone injuries/fractures (KBF). However, the effect of such training regimens on energetic cost is unknown. Evaluating the effects of training regimens on energy expenditure is critical to determine whether pullets have sufficient energetic capacity to support growth, development, and subsequent egg production at sexual maturity. The doubly labeled water (DLW) method is commonly used to quantify daily energy expenditure (DEE, kJ/day) in freely moving birds. Since chicken flight muscles are anerobic, and support short, explosive flights, we hypothesized that CFD training would not increase mass-adjusted DEE measured after 10 weeks of CFD training, regardless of improvements in maneuverability in their home environment. In this study, 42 white (W) and 46 brown (B) feathered birds were housed in 12 mixed breed floor pens. Birds were assigned to the trained (T, W: n=19; B: n=24) or untrained group (U, W: n=23; B: n=22), and the distribution of birds in each group was balanced across pens. CFD training began at 6 days of age and continued until 10 weeks of age (WOA) for a total of 46 days. Pullets had training 5 days per week followed by 2 rest days. Training occurred within the pen, and all birds were handled during training sessions. Untrained birds remained in the pen during training sessions to provide social motivation to birds performing CFD. Over the training period, the number of CFDs increased (1 to 15) and rest between successive CFDs decreased (45 second to 5 seconds). Pens were equipped with perches no higher than 15cm during the training period. DEE of W pullets (T: n=19; U: n=23) was measured using DLW over 47 hours at the completion of CFD training (10WOA), and at 19WOA. Pullets received a dose of stable, non-radioactive isotopes deuterium (^{2}H) and oxygen-18 (^{18}O) via intramuscular injection. At one and 48 hours post-injection, blood samples were collected, and the relative elimination of ^{2}H (H2O loss) and ^{1}□O (H2O + CO_2 loss) was used to calculate CO_2 production and thus DEE. Experimental protocols were approved by the University of Guelph Animal Care Committee (Animal Utilization Protocol #5173). DEE outcomes at 10WOA were obtained from the age-specific least-squares means of a repeated-measures generalized linear mixed model (SAS) using a log normal distribution. Training status, age, and average mass (kg) were used as predictors, and pen as a random effect. Only results from 10WOA are presented here. Body mass had a significant positive relationship with DEE ($F=24.11$, $p<0.0001$); therefore, heavier birds expended more energy per day. CFD trained pullets had no increase in daily metabolic load at 10WOA (T=774.78 ± 10.03kJ/day vs. U=779.81 ± 9.37kJ/day, $F=0.17$, $p=0.6791$), indicating that this training regimen does not incur extra energy costs upon completion. Practically, this supports an approach in which CFD activity during rearing could be used to build maneuvering skill without compromising energy available for growth or increasing daily energetic requirements. We are presently examining the effects of CFD training on the prevalence and severity of KBF and maneuvering capacity to test the efficacy of CFD training on the improvement of welfare. Results of such analyses will be presented at the conference.

Isolation of bovine mesenchymal stroma cells from hair follicles as an in vitro model for studies on bovine digital dermatitis

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The principles of the 3Rs (replace, reduce, and refine) provide ethical guidelines for experimental research involving laboratory animals. In our current study, we aim to establish a non-invasive method for isolating bovine mesenchymal stroma cells (MSC) from plucked hair follicles to provide an in vitro cell model. Our experimental approach is novel, as common sources of MSCs in livestock include bone marrow, adipose tissue, or hair follicles obtained from skin biopsies, all of which rely on invasive extraction methods. Our research focuses on the bovine infectious hoof disease digital dermatitis (DD), which has a multifactorial aetiology and a polygenic influence on susceptibility. Through a genome-wide association study, we identified two polymorphisms with significant effects on susceptibility, indicating that specific genotypes were significantly more likely to be affected and to develop chronic lesions. Deeper insights into potentially altered inflammatory processes due to the analysed polymorphisms near CMPK2 (BTA 11) and in ASB16 (BTA 19) could help to develop more animal-specific immunomodulatory therapies. For planned studies on potentially altered cell behaviour and/or cell-cell-communication due to the genotype variants, we intend to use different cell types, such as MSCs, which play an important role in immunological processes during bacterial infections. Additionally, assessing the ability of MSCs to differentiate into keratinocytes is included and may enable us to investigate skin-associated signalling based on this important cell type. To date, MSC cultures from four cattle with different genotype constellations regarding DD have been established. Hair follicles were cultured in vitro with feeder cells until MSC outgrowth occurred after 4-12 weeks. For reliable identification of MSCs, a characterisation analysis was performed based on the recommendations of the International Society for Cellular Therapies (ISCT). Our cells exhibit the typical spindle-shaped morphology, grow adherently on plastic culture dishes, and proliferate stably (over 20 passages). Phenotypic characterization for expression of MSC-specific surface markers was performed with Flow Cytometry. The antibodies successfully labelled bovine MSC surface markers (CD29, CD44, CD90), while not (CD14, CD45) or moderately (CD34) labelling the negative markers. Additionally, expression of the stem cell marker SOX2 was detected via immunofluorescence staining. MSCs should exhibit the potential for chondrogenic, osteogenic, and adipogenic in vitro differentiation. Differentiation into all three lineages was demonstrated by detection of proteoglycans (Alcian Blue staining), calcium phosphate (Alizarin Red staining), and perinuclear granules with expression of the adipogenic marker PPARγ by immunofluorescence staining. Further isolation of MSCs for more comparative investigations regarding functional effects of the genetic variants is ongoing. Analyses will include transcriptome analyses, assays on physiological cell behaviour, communication potential, and cellular viability. Our innovative method for non-invasive isolation of MSCs provides animal-specific in vitro cell models for research on immunological issues in accordance with the 3R principle. Further studies on the applicability of our results to other livestock species are planned.

In vitro digestion in swine: how close is it to in vivo digestion? – A Systematic Review

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Digestibility analysis in pigs is traditionally performed through in vivo experiments using indigestible markers, which require animal use, large amounts of feed, and labor. In vitro digestion has emerged as a faster and more cost-effective alternative aligned with the 3R's principles, although discrepancies with in vivo results may occur due to limitations in simulating physiological digestive conditions. Compare the methodologies and results of in vitro and in vivo digestibility studies in pigs through a systematic review, evaluating their reproducibility and reliability. A systematic search was conducted in PubMed, Scopus, and Web of Science without date restrictions, including peer-reviewed articles in English. The review followed the PICo framework, considering pigs as the population, in vitro digestion as the interest, in vivo digestion as the comparison, and the reliability of in vitro methods as the outcome. Based on this, the following search string was constructed: ("pig" OR "swine" OR "sow*" OR "growing pig*" OR "weaned pig*" OR "nursery pig*") AND ("in vitro digestion" OR "in vitro" OR "simulated digestion" OR "enzymatic digestion") AND ("in vivo digestion" OR "in vivo" OR "ileal digestib*" OR "total tract digestib*") AND ("nutrient absorption" OR "nutrient digestib*" OR "digestib* coefficient" OR "apparent digestib*" OR "true digestib*"). After duplicate removal, studies were screened by title, abstract, and full text, including only original articles comparing in vitro and in vivo digestibility in pigs with clearly described methodologies. The search identified 332 records, with 207 non-duplicate articles screened by title and abstract and 59 assessed in full text, resulting in 14 articles (16 experiments) included for data extraction. The methodology of Boisen and Fernández was the most frequently used (42.86%). Simulated digestion conditions varied widely, with stomach phases of 1.5–6 h, intestinal phases of 4–18 h, and temperatures of 39–40 °C. Missing methodological information was common, particularly regarding in vivo trial duration (35.71%), animal production phase (28.57%), and reagents used (21.43%). Regarding in vivo trials, the studies reviewed addressed four different methodologies for determining digestibility, including the use of ileal cannulas, fecal sample collection, ileorectal anastomosis, and ileal digesta collection at the time of slaughter. Based on these procedures, different digestibility parameters were estimated across the studies. Among them, apparent ileal digestibility was the most frequently reported, followed by in vitro digestibility and total tract digestibility. Other parameters, such as ileal + fecal digestibility, fecal digestibility, ileal digestibility, in vitro protein digestion kinetics, digestible energy, and ileal/total tract disappearance, were reported less frequently. In vitro digestion does not fully replicate in vivo digestion in pigs, but it can approximate fecal digestibility under specific conditions. The degree of reliability depends on the chosen protocol and the study objectives, since no universal method applies to all experimental contexts. Thus, in vitro systems should be regarded as complementary tools rather than definitive substitutes for in vivo approaches.

Development of Ovine Hepatic Organoids: A Powerful In Vitro Platform to Reduce the Number of Experimental Animals Used in Metabolism and Nutrition Assays

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The liver plays a central role in coordinating metabolic processes that determine growth, health, and production efficiency in livestock species. In ruminants, hepatic metabolism is uniquely adapted to support glucose homeostasis, lipid partitioning, and nitrogen utilization under conditions imposed by ruminal fermentation, making predictive experimental models essential for studying nutrient-mediated regulation of liver function. However, conventional two-dimensional hepatocyte cultures fail to reproduce the structural organization and functional complexity of native liver tissue, limiting their translational value. In this study, we established and characterized three-dimensional hepatic organoids derived from ovine liver progenitor cells as an advanced in vitro model to investigate ruminant hepatic metabolism. Ovine hepatic organoids were generated from the liver of a male Assaf lamb and characterized by histological and immunofluorescence analyses, followed by transcriptomic profiling using RNA-seq and functional enrichment analysis. Finally, in order to assess the metabolic responsiveness of these hepatic organoids, DL-methionine and/or betaine were supplemented before measuring by RT-qPCR the expression of genes encoding key metabolic enzymes (e.g., CPT1A and PDH1) and ribosomal proteins representative of cellular proliferation (e.g., RPL22L1). According to the results obtained, ovine hepatic organoids formed spherical epithelial structures with defined apical–basal polarity, intact tight junctions, albumin expression, and intracellular glycogen accumulation, indicating preservation of key hepatic features. Comparative transcriptomic analysis revealed extensive overlap between organoids and native liver tissue, with the majority of expressed genes shared between both systems, supporting the conservation of core cellular programs. Nevertheless, differential expression and functional enrichment analyses demonstrated that hepatic organoids exhibit a transcriptional profile biased toward cell proliferation, protein synthesis, and structural remodeling, while genes associated with mature liver metabolic functions, immune responses, and systemic homeostasis were relatively downregulated, which is consistent with a partially differentiated or progenitor-like state. Finally, combined DL-methionine and betaine treatment significantly increased the expression of the CPT1A gene associated with mitochondrial beta-oxidation, indicating a synergistic metabolic response of both compounds and demonstrating the metabolic responsiveness of these ovine hepatic organoids. This work represents the first report of ovine hepatic organoids and establishes a foundation for their use as predictive in vitro platforms to study hepatic metabolism, nutrient–gene interactions, and feed additive strategies in ruminant livestock.

Dynamics of Glutathione Redox Status in Poultry: Impact of Age and Handling Stress

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Commercial poultry production exposes birds to multiple stressors that often triggers oxidative stress, impairing health and performance. Reactive oxygen species (ROS) play a dual role: essential for redox signaling and immune defense at physiological levels, yet harmful when overproduced or when antioxidant defenses are weakened. Glutathione (GSH), the most abundant non-protein thiol, is central to maintaining cellular redox balance, regulating gene expression, and protecting organelles such as mitochondria and nuclei. The GSH/GSSG ratio is a key indicator of redox potential and oxidative status. This work aimed to evaluate GSH redox dynamics in two contexts: (1) growing broilers without any specific challenge and (2) adult laying hens subjected to repeated handling stress (weekly manipulation (n=3) for blood sampling over 2 weeks). In the first study, plasma GSH and GSSG were analyzed in 30 Ross 308 broilers between day 14 and day 32. Results revealed a significant increase ($P<0.01$) in the GSH/GSSG ratio (+37%) over time, despite reductions in both GSH (-6%; $P=0.156$) and GSSG (-30%; $P<0.01$) concentrations, suggesting adaptive antioxidant responses during growth. In the second study, weekly blood sampling of 57 laying hens demonstrated a progressive rise ($P>0.10$) in oxidized glutathione (GSSG), a tendency for a reduction of GSH between d7 and d14 resulting in a non-significant decrease in the GSH/GSSG ratio after one and two weeks. However, mobilization of antioxidant defenses did occur again since laying performance was negatively impacted ($P<0.05$) over 2 weeks. Under stress, GSH acts as a critical redox buffer, detoxifying ROS and supporting GSH-dependent enzymes such as glutathione peroxidase, thereby preserving protein thiols and signaling integrity. Disruption of GSH homeostasis and abnormal protein S-glutathionylation are linked to mitochondrial dysfunction, apoptosis, and impaired immunity, ultimately compromising productivity. These findings highlight the importance of GSH-driven antioxidant strategies to mitigate oxidative stress in poultry systems.

Ex vivo cultivation of primary bovine and porcine fat cells to optimize and refine animal tests

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Adipose tissue development and lipogenesis are critical factors for livestock production efficiency and meat quality. Adipocyte-related investigations typically use in vitro cell lines, such as 3T3-L1 and preadipocytes, which rely heavily on adipogenesis efficiency and neglect substantial discrepancies between differentiated adipocytes and native adipocytes in living organisms. In addition to cell models and different species, different white adipose depots, such as intra- and intermuscular, subcutaneous, perirenal, and mesenteric, also show distinct gene expression profiles and metabolic characteristics. Although in vivo experiments provide physiological relevance, they require extensive animal use and are influenced by systemic variables. Ex vivo primary adipocytes retain native cellular properties and therefore represent a promising intermediate model that may bridge the translational gap and contribute to the refinement and reduction of animal testing. Our study aimed to optimize a culture system for floating primary white adipocytes to facilitate adipocyte maintenance, imaging, and functional analysis. We designed a culture system based on the classical ceiling culture approach to adapt conventional cell culture dishes and multi-well plates. Primary white adipocytes were isolated from bovine and porcine subcutaneous fat for ex vivo culture. Antioxidant supplementation in culture medium, such as 2-Mercaptoethanol, was introduced to enhance cell survival following enzymatic isolation and ex vivo cultivation, a critical step at which adipocytes are particularly vulnerable. Comparative analyses were performed alongside established in vitro models, including 3T3-L1 and preadipocyte-differentiated adipocytes, to evaluate morphology including lipid droplet and live mitochondria staining, gene expression and adipokines secretion, and experimental responsiveness including β-adrenergic agonists and insulin stimuli. Our improved culture protocol enables stable maintenance of ex vivo primary white adipocytes in standard culture formats and supports feasible microscopic imaging and downstream assays. Primary adipocytes, which typically exhibit limited survival after isolation, remained viable for several days under optimized antioxidant-supported cell culture conditions. The larger size and appearance of unilocular lipid droplets were more consistent with in vivo adipocyte physiology than those observed in cell model-derived adipocytes. Notably, the ex vivo adipocytes also keep the ability to dedifferentiate, proliferate, and expand. In conclusion, the optimized ex vivo primary adipocyte cultivation system provides a physiologically relevant and experimentally accessible platform that preserves depot-specific uniqueness. This enables investigation of distinct effects of nutritional or pharmacological substances on adipocytes and offers the potential to develop strategies to control fat metabolism and deposition. Finally, more physiologically similar ex vivo experiments can help to further optimize animal experimental protocols. This work will contribute to the 3R principles by improving in vivo experimental predictability and potentially decreasing the number of animals used in adipocyte-related research.

Targeted sequencing approach for equine microsatellites (Simple Tandem Repeats, STRs) and Single Nucleotide Polymorphisms (SNPs) genotyping to enable transition from STR to SNP-based Equine parentage verification

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Equine STRs based parentage verification has been extensively used by the horse breeding industry for decades. For this purpose, the International Society for Animal Genetics (ISAG) established a recommended Equine STR panel which currently consists of 12 markers in the core panel. These markers are used by laboratories and studbooks worldwide and enable the exchange of DNA profiles across borders. In 2023, ISAG established a recommended Equine SNP-panel for parentage verification. Many Equine Studbooks and laboratories are considering a transition to equine SNP-based parentage verification. The advantages of an SNP-based approach are that it lends itself to multiplexing thus enables simultaneous genotyping of markers associated with genetic disorders/traits together with breed and parental identification, and by creating a high-density SNP-panel, provides deeper insight into genetic diversity and the development of robust genomic breeding values. This study aims to develop and evaluate a multiplex targeted sequencing panel combining STR and SNP markers for equine parentage verification on the AgriPlex PlexSeq Platform. The panel includes the ISAG STR-markers, ISAG SNP-markers, and a set of markers for genetic disorders and characteristics. Currently the developing panel provides ~90% call rate and the objective is to further elevate this value and to proceed to test the panel on a set of reference samples that will enable to evaluate concordance with STR genotyping, error rates, and parentage exclusion probabilities. This multiplexed targeted sequencing panel is aiming to be an intermediate tool that will facilitate the gradual transition of Equine Studbooks and laboratories from STR-based to SNP-based parentage verification.

Frequencies of known genes of interest in French horses genotyped using a 60K SNP Chip

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The Axiom GENOFIL 60K SNP array (60,752 SNPs) was developed by the French equine sector to enable large-scale genomic deployment across French horse populations. This array has been designed to meet multiple objectives, including the analysis of major genes of interest. Given the high diversity of equine breeds in France, objectives differ substantially among Breeding Organizations. Consequently, the relevance of major genes varies across populations, particularly for health- and performance-related traits. Access to genotypes at key loci enables population-level monitoring of allele frequency dynamics and provides a framework for adapting breeding strategies. This study aimed to estimate allele frequencies of major genes of interest across French horse populations using the GENOFIL 60K SNP array. 5,206 horses genotyped with these array were analysed and then divided into seven groups: Thoroughbreds (n=1,250), Trotters (n=397), Arabians (n=419), Sport horses (n=1,520), Ponies (n=1,386), Draft horses (n=148), and Local breeds (n=86). We investigated 84 SNPs associated with 34 major genes of interest, classified into four categories: monogenic (n=16) and polygenic (n=3) health traits, performance traits (n=3) and coat colour traits (n=12). Here we present selected results for major health and performance genes. Polymorphisms were identified in eight genes belonging to the first group. The PLOD1 mutation (Warmblood Fragile Foal Syndrome WFFS) showed variable frequencies across populations, with a moderate proportion of heterozygotes in Sport horses (7.4%). It was also unexpectedly detected in Thoroughbreds (3.6%), while remaining low in Ponies (1.5%) and Arabians (0.2%). As anticipated, MUTYH (Cerebellar Abiotrophy CA) predominantly affected Arabians with 7.6% of heterozygotes, while CLCN1 (Myotonia Congenital MC) was mainly observed in Ponies (2.2% of heterozygotes). In contrast, the GYS1 gene (Polysaccharide Storage Myopathy PSSM) showed a high prevalence in Draft and Local horse populations, with high proportions of heterozygous individuals (30.2% and 45.9%, respectively) and notable levels of homozygotes (4.7% and 12.8%). For DMRT3, as expected, the AA genotype was highly prevalent in Trotters (69.4%), where it characterizes horses with a natural aptitude for trotting, whereas only the CC genotype, associated with galloping ability, was observed in Arabians and Thoroughbreds. The MSTN gene variant determines the preferred racing distance in gallop races. It showed a more balanced distribution of genotypes in Thoroughbreds, with individuals represented across short-, middle- and long-distance performance profiles (25.1%, 45.4% and 29.6%, respectively). The LCORL gene revealed clear size-related patterns across populations. The small-size genotype predominated in Arabians (95.9%), Ponies (84.7%), Thoroughbreds (64.4%) and Local breeds (64.3%), whereas the large-size genotype was nearly fixed in Draft horses (98.0%). In contrast, Sport horses displayed a more even distribution of size genotypes (32.7% large, 53% medium and 14.3% small). With the perspective of transition to SNP-based parentage control, the GENOFIL 60K array will provide genomic data across French horse populations. Breed-level summary statistics generated from these data offer stud-books robust indicators to monitor population dynamics, while individual-level results deliver actionable information to breeders. These results highlight substantial variation in allele frequencies across breeds, reflecting both historical selection and the distribution of inherited disorders.

Genetic diversity in Dutch horse studbooks based on collaborative pedigree analyses

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Maintaining genetic diversity in domestic horse populations is important to ensure the long-term health, performance, and adaptability of these populations. In the Netherlands, 47 national and international horse breeds are represented, each shaped by its own history of breeding goals, breed popularity, and (inter)national exchange. This study aimed to assess genetic diversity across Dutch horse studbooks using pedigree data and to identify key drivers of inbreeding through a collaborative approach with studbooks. Within the collaborative project 'BehoudenPaard' ("Conserved Horses"), workshops were conducted with studbook representatives to jointly analyse pedigree data from multiple breeds and to build capacity for long-term monitoring of genetic diversity within these populations. These breeds include, among others, Haflinger, Fjord, Dutch miniature, Friesian, Dutch warmblood, Shetland ponies and Welsh and Cob. We first focused on cleaning the pedigrees. Pedigree data were first curated using a custom-developed Streamlit application (https://pedigreecleaner.streamlit.app/) to identify and correct common inconsistencies, including duplicate records, implausible parentage, circular references, and unusually high reproductive output. Subsequently, pedigree analyses were performed to evaluate temporal trends in (1) population size, (2) parental contributions, (3) generation intervals, (4) pedigree completeness, (5) inbreeding and kinship coefficients and their rates of change, (6) founder representation, and (7) population structure in breeds with multiple lines or subpopulations. We recognized several drivers for (too) high inbreeding rates in some of the populations, including a small census size, a (relatively) small breeding population, a "popular sire effect", and the direct replacement of breeding animals by their offspring. Populations varied substantially in these aspects, for example in number of foals born per year ranging from 48 for Groninger, 258 for the Dutch Draught horse to almost 5,000 for Dutch warmblood showjumping horses in 2024. Inbreeding rates also varied across (sub)populations and over time, with historical inbreeding rates reaching in some cases up to 4.5% per generation, and more recent inbreeding rates varying from -0.95% to 2.2% per generation. Here, we will present the variation in genetic diversity metrics across studbooks as well as common threats that negatively affect genetic diversity. We will organise additional workshops on population-specific genetic management strategies, the incorporation of DNA test results into selection schemes, and provide tools for communication of lessons learned to breeders and studbook members to create support and commitment in the long term. These results highlight substantial variation in genetic diversity across Dutch horse studbooks and identify key drivers of inbreeding, including small effective population size and the widespread use of popular sires. The collaborative approach proved effective in increasing awareness and supporting the implementation of sustainable genetic management strategies.

Genomic inbreeding level of the Coldblooded trotter

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The Coldblooded trotter is a breed used for harness racing in Sweden and Norway. It was officially split from the North Swedish horse and the Norwegian Dole horse in the 1960s. Due to extensive exchange of breeding animals between the countries, a joint studbook was established in 2000. Intense selection for racing performance and the use of a limited number of popular stallions have led to increased relatedness among individuals in the population. For horses born in 2021, the inbreeding level based on pedigree information (FPED) has been estimated at 0.084, while the inbreeding rate per generation is 0.012. Previous studies have also reported signs of inbreeding depression in racing performance and fertility. Furthermore, the annual number of foals born has more than halved since the 1990s, raising concerns about loss of genetic diversity. The aim of this study was therefore to estimate the genomic inbreeding level of Coldblooded trotters for strategic management of the genetic resources in this breed. A subset of 81 breeding sires was genotyped with the Affymetrix 670K chip array, where 285,266 SNPs passed quality control. Runs of homozygosity (ROH) were analyzed in PLINK v1.9 with parameter settings based on within-ROH inbreeding developed by Wage et al. (2025). Parameters were set to the following: 50 consecutive homozygous SNPs with the minimum length of ROH set to 500 kb. One missing and zero heterozygote SNPs were allowed per window with a sliding-window threshold of 0.05. The maximum gap between SNPs was set to 1000 kb, and the minimal SNP density within ROH to 1 per 50 kb. A total of 12,346 ROH were retrieved and distributed across the following bin lengths: 0.5-2Mb: 42.4%, 2-4Mb: 27.1%, 4-8Mb: 21.2%, 8-16Mb: 8.3%, and >16Mb: 1.0%. The inbreeding level based on ROH (FROH) ranged from 0.01 to 0.21, with an average inbreeding level of 0.12. The correlation between FROH and FPED was estimated at 0.46. These preliminary results suggest recent inbreeding in the population of Coldblooded trotters. The low correlation found between FROH and FPED indicates that further evaluation is needed to determine the best criteria for managing genetic diversity. With additional genotyped horses, inbreeding depression in racing performance traits and founder-specific inbreeding effects will be studied. The results from this study will aid the breeding organization in developing strategies to maintain the genetic diversity of the Coldblooded trotter.

Size matters – characterisation of candidate causal variants for withers height in horses

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Withers height is a major selection criterion in horse breeding. Beyond its influence on overall appearance, it is associated with health and performance and plays an important role for the classification and market value of horses. Hence, uncovering the genomic basis of withers height is of considerable scientific interest. In a previous study (Reich et al., 2024), a sequence-based GWAS for withers height using imputed data from 2709 German warmblood horses revealed two genome-wide significant association signals on ECA1 and ECA3. Further analysis of the QTL regions resulted in the identification of several candidate causal variants, which were investigated in more detail in the present study to validate their potential impact on withers height and extend the analyses to additional breeds. The three most promising variants – a nonsense mutation in LCORL and a missense mutation in NCAPG in high linkage disequilibrium (ECA3), as well as a missense mutation in the TET1 gene (ECA1) – were genotyped by RFLP or Sanger sequencing in over 250 horses from different breeds. Additionally, genotypes obtained from publicly available sequence data from 317 horses compiled in a previous study (Reich et al., 2022) were considered in the analyses. Genotype data from the included German warmblood horses confirmed allele frequencies close to 0.5 for the LCORL and NCAPG variants, as observed in the previous study using imputed sequence data, and corroborated their very high linkage disequilibrium. The mutant alleles, which were associated with increased withers height, occurred at slightly higher frequencies in other warmblood breeds and Standardbreds, while they were less common in Thoroughbreds and Franches-Montagnes horses (~0.10-0.15). Interestingly, they were very rare or absent in different pony breeds, including German riding ponies, Haflinger and Shetland ponies, as well as Arabians and Quarter Horses. On the other hand, they occurred at very high frequencies (close to 1) in different coldblooded breeds. However, for one light and small draft horse breed (Black Forest horses), the mutant alleles were considerably less common (~0.18) than in the other coldblooded breeds. The TET1 mutant allele occurred at rather low frequencies in warmblood horses and was more prevalent in coldblooded breeds (~0.20-0.25), while it was absent in most other investigated breeds, suggesting a potential impact on the body type of horses. Furthermore, linear regression on over 110 genotyped horses with available phenotype data confirmed the statistically significant association of the LCORL, NCAPG and TET1 variants with withers height both across and within different horse breeds. Likewise, variants in all three of these genes have previously been found to be associated with body size and mass traits in several other species. Taken together, these findings provide strong evidence for the involvement of LCORL, NCAPG and TET1 in the genetic architecture of withers height in horses. The near fixation of the 'small' and 'tall' alleles in pony and coldblooded breeds, respectively, together with the strong association in warmblood horses and consistent findings in other species, highlights the LCORL nonsense mutation as the most promising candidate causal variant. The observed allele frequency patterns across breeds likely reflect historical selection for body size and suggest potential for future genomic selection strategies. However, additional investigations, including gene expression and functional analyses, will help further validate the role of the identified variants in withers height in horses.

Inter- and intra- rater reliability of linear scoring in warmblood breeding

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Reliable phenotypes are a key prerequisite for successful animal breeding programmes and the advancement of genomic applications. However, subjective phenotypic assessment may limit genetic progress and the power of genome-based analyses. In warmblood horses, conformation is commonly assessed using linear scoring. Each score on the linear scoring scale describes one trait within its biological extreme expressions. Despite its important role in the selection process of young breeding horses, the reliability of linear scoring in the context of warmblood breeding has not been sufficiently quantified. In this study, we investigated inter- and intra-rater reliability of linear scoring for conformation traits in German warmbloods. Eleven professional equine breeding experts (e.g. breeding judges) completed an online questionnaire, scoring 17 linear conformation traits in 34 warmblood stallions using side-view images. Participants were asked to repeat the questionnaire after approximately one month. The images originated from the archive of the Hanoverian State Stud in Celle and showed warmblood stallions born between 1929 and 1993. Moreover, these historic images ensured the representation of a wide variety of phenotypes under standardised environmental conditions for all raters. Inter- and intra-rater reliability was assessed using intraclass correlation coefficients (ICCs). Permutation tests were performed for both rounds of the inter-rater analysis and the intra-rater analysis. To determine whether rater scoring was superior to random assignment, all values in the horse × judge matrices were shuffled within each trait separately to eliminate any horse-judge structure while preserving the empirical score distribution. Overall, inter-rater reliability was lower than intra-rater reliability. Inter-rater ICCs ranged from 0.3 to 0.56 across rounds and traits. Intra-rater reliability varied between raters and traits, ranging from poor consistency (ICC = -0.36) to excellent consistency (ICC = 0.90) between rounds. Trait-specific patterns were identified across both types of analysis: the trait Head achieved moderate to good inter- and intra-rater agreement, while traits requiring closer assessment of underlying anatomical structures (e.g. Shoulder Length and Carpal Joints) showed ICCs close to zero. Randomly shuffled horse × judge matrices revealed that most raters outperformed random scoring. Occasionally negative ICC values were observed in the intra-rater analysis indicating that variance between repeated ratings is higher than the phenotypic variance among subjects. Mean linear scores were close to zero, and mean absolute deviations between 0.32 and 0.90 indicated limited use of the -3 to +3 scale. The results of this study show that linear scoring by experts captures non-random phenotypic information. Still, the reliability of assessments, in particular between raters and for more complex traits, is on average low. Enhancing the reliability of linear scoring in warmblood breeding will require revision and standardisation of trait definitions, regular exchange among experts, and extended training of judges to improve consistency and ultimately increase the value of phenotypes for practical breeders or genomic applications.

Genetics of racing performance across different track conditions in Thoroughbred horses in France

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The objective of the study was the estimation of genetic parameters of racing performance and the genetic characterization of the diversity of careers in flat horse racing regarding tracks condition. France Galop provided racing records spanning 2000 to 2024, encompassing 709,381 individual race results from 84,204 Thoroughbred horses in 67,310 flat races on turf track. Performance criteria were the logarithm of earnings (LE) for each race and each horse – either actual or simulated by halving the amount for each successive rank for non-winning horses – and career start status (binary variable: 1 if the horse started at least once, 0 otherwise). Performance was analyzed by track condition based on penetrometer measurements, with four categories: very firm to good, good to soft, soft, very soft to very heavy. Among the horses, 67% raced on very firm to good, 63% on good to soft, 60% on soft, 68% on very soft to very heavy track. The analysis used a multi-trait animal model with LE as linear trait and start status as threshold trait, and including age, stall number (both for LE only), combination of sex, year and country of birth, and inbreeding coefficient (mean of 1.98%, calculated using the whole pedigree with 8.2 generations on average, and divided into 8 classes) as fixed effects, and trainer (n=3,335), animal additive genetic value (180,207 horses in pedigree), and permanent environment as random effects. Analysis was performed by Markov chain Monte Carlo Gibbs sampling using the TM software. Both extreme inbreeding levels had negative effects on start status and LE for all track conditions. Compared with 1.0–1.5% level, very low (<0.5%) and high (>5%) inbreeding resulted in −0.08 to −0.02 and −0.05 to −0.03 phenotypic standard deviation (SD) units for start status, respectively. Similarly, very low and high inbreeding resulted in −0.03 to −0.05 (<0.5%) and −0.07 to −0.11 (>5%) phenotypic SD units for LE, across track conditions. Trainer proportion of variance was 33 to 39% for start status and 8 to 9% for LE. Heritability estimates (standard errors <0.01) ranged from 0.11 to 0.13 for start status and from 0.09 to 0.10 for LE. These results reveal that the effect of the trainer on participation is more important than genetic effect. However, the effect of the trainer on success (LE) is roughly as important as genetic effect. Genetic correlations (standard errors <0.05) among start status were positive and high (0.64 to 0.80) as they were among LE (0.84 to 0.97), revealing that there is no genetic predisposition in participating on a specific track condition. In addition to that, genetic correlations between start status and LE were close to zero, varying between -0.30 and 0.04, revealing that participating on a given track condition and LE on the same or another track condition are genetically close to independent. Main results enabled us to conclude that participation on different track conditions depends more on trainer than on genetics and constitutes a genetically single trait. Furthermore, successes on different track conditions are based on the same genetic foundations. These results suggest a shared genetic architecture of racing performance across track conditions, with participation being primarily influenced by trainers. Similar analyses were made across race category and distance.

Revising selection criteria for endurance performance in Spanish Horses

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The Spanish Arabian Horse Breeder Association (AECCA) has developed a breeding programme with the aim of improving endurance performance in Spain. There are young horse competitions (YHC) designed specifically for early performance control, where the emphasis is on finishing the race rather than animal's final ranking position. These competitions are for horses aged between four and seven years old. The selection criteria for YHC are based on three traits combined in a genetic index: race time (RT, defined as the time taken to complete the race, minus the time taken to pass the veterinary control); recovery time (REC, defined as the time taken to pass the veterinary control); and placing (P, defined as a categorical trait indicating whether the horse finished the race or not). Genetic and phenotypic trends have recently been analysed, revealing no genetic trend for race time. When analysing phenotypic trends in race time, it appears that there is a limit to how fast horses can become, suggesting that genetic improvement may be slow. Furthermore, it seems that genetic progress is focused more on recovery time than on race time in these young competitions. If the speed is increased through selection, there may be problems in passing the veterinary control, so it may be preferable to maintain the race time. The objective of this study was to evaluate the inclusion of a new selection criteria in the genetic evaluation of endurance horses. We analysed the time per kilometre (TK) and the speed (S). The data set contained 4895 records from YHC. The pedigree included 17254 individuals (59% of which were Arabian horses). To evaluate the two new traits, univariate and multitrait models were run, including TK or S with REC and P. The TK and S models included sex (male, female, gelding), age (five levels), distance as covariate (linear and quadratic) and event (456 levels) as fixed effects, and rider as additional random effect. REC and P were studied using the same model but without including distance. The heritabilities for these new analysed traits were 0.12 (0.02) for TK and 0.09 (0.03) for S. This last heritability was lower than the value found for RT (0.12 (0.02)). Heritabilities for REC and P were 0.27 (0.03) and 0.04 (0.02), respectively. The rider effect variance ratio was 0.09 (0.02) for TK, 0.10 (0.02) for S, 0.09 (0.02) for REC, but was not different from zero for P. Regarding genetic correlations with other traits, it was shown that none of the trait pairs had a genetic correlation that was different from zero. It seems that either TK or S could replace RT. Speed could be important in higher level races where the horses are well trained and there is no speed limit. This could improve the genetic trend for the time taken to complete the race in future, but studies of older horses in higher level competitions are also needed.

Early genomic selection for fertility in Pura Raza Española horses using array equigene

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Fertility is economically critical in horse breeding, yet genetic improvement remains challenging due to low heritability and late phenotypic expression. Traditional evaluation requires waiting 4-6 years until first foaling, followed by additional years to assess lifetime performance, creating generation intervals of approximately 10 years. The objective of this study was to evaluate whether genomic information enables early identification of animals with different reproductive potential before phenotypes become available. We analyzed 4,750 Pura Raza Española horses genotyped with the Axiom EQUIGENE 90K SNP array (81,543 SNPs post-quality control), with reproductive phenotypes from 47,502 breeding records for seven fertility traits: Reproductive Efficiency, Age at First Foaling, Age at Last Foaling, Total Foaling Number, Average Interval Between Foalings, Interval between First and Second Foaling, and Productive Life. Heritability estimates ranged from 0.07 (Interval between First and Second Foaling) to 0.35 (Age at Last Foaling). Genomic breeding values were estimated using single-step genomic methodology integrating pedigree and marker information, providing genetic predictions for all animals including young individuals without phenotypes. Animals were grouped according to their genomic breeding values to evaluate whether early genomic predictions correspond to subsequent reproductive performance. Young animals in the top group for genomic merit (highest 20%) were compared to those in the bottom group (lowest 20%) to examine differences in actual fertility outcomes when phenotypes later became available. Results showed that genomic predictions successfully distinguished between animals with different reproductive potential. Animals with high genomic merit at weaning showed better subsequent fertility performance, including earlier age at first foaling, higher total foaling numbers, and better reproductive efficiency compared to animals with low genomic merit. The ability to rank animals by reproductive potential at weaning (6-8 months of age), years before traditional evaluation becomes possible (5-6 years), enables earlier selection decisions. Breeders can identify superior breeding prospects and make culling decisions for animals with poor genetic merit at weaning rather than investing resources in raising them to breeding age. Implementation of genomic selection at weaning instead of waiting for phenotypic maturity could reduce generation intervals from approximately 10 years to 6-7 years, representing a 35-40% reduction and proportional acceleration in genetic progress for fertility traits.

Genomic breeding values for sport performance in German riding horses

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National genetic evaluation for sport performance in riding horses in Germany is carried out annually on behalf of the German Equestrian Federation (FN). Since 2019, the system includes the traits highest level achieved in dressage (HLD) and show jumping (HLJ), proposed as target traits for future routine genomic evaluation. The aim of this study was to develop the prototype of single-step genomic evaluation for HLD and HLJ following the approach used for the new routine genomic evaluation for linear conformation, movement and jumping traits. The national genetic evaluation considers starts in dressage and show jumping competitions and is based on all national competition data and additionally international competition data of German horses. For each horse, HLD and HLJ are determined under the precondition of at least three starts in the respective discipline. For this study, HLD and HLJ data were made available by the FN for 348,471 horses registered by one of the German studbooks for riding horses belonging to genomic consortium. In the genomic evaluation system, this phenotypic data was used together with genome-wide SNP genotypic data (80K / Equine80select) and pedigree data over four ancestral generations. This resulted in a total system size of 818,340 horses analyzed with the single-step method using Mix99 software and the same model as in the national genetic evaluation. The following fixed effects were included: sex (mare, stallion or gelding), year in which HLD or HLJ was first achieved (calendar year, 1996 – 2025), age of horse at last competition start (4-5, individual years from 6 to 14, 15 and older). Estimated genomic breeding values (gEBV) were standardized to a mean of 100 and genetic standard deviation of 20, with basis defined by the 6- to 9-year-old horses with phenotype (N = 21,958 horses born 2017 – 2019).Results from the national genetic evaluation (EBV) were available for published stallions and active broodmares of the studbooks from the genomic consortium and were used to determine Pearson correlation coefficients between gEBV and EBV. High correlations of 0.97 for HLD and 0.96 for HLJ were found, indicating no major re-ranking. With a growing number of horses with sport-related phenotypic information and genotypic data, reliability of gEBV is expected to increase and improve discrimination between individual horses' genetic potential for performance in dressage and show jumping. Currently, it is mainly older stallions fulfilling publication criteria for EBV for HLD and HLJ, and EBV for mares often remain vague. In the future, sport horse breeders in Germany will increasingly benefit from early implementation of routine SNP genotyping giving early access to gEBVs for a growing range of traits. Calibration and validation of the presented prototype will prepare for complementing the genomic support of selection and mating decisions of sport horses in Germany.

Discipline specialization and its impact on genetic trends followed by genomic breeding values for conformation, gaits and jumping in sport horses in Germany

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Within the framework of breeding programs of the studbooks, sport horse breeding is shaped by expectations of riders and concepts of breeders. Studies have shown that the roles of the two main disciplines of riding sport, dressage and show jumping may have changed over time, with specialization understood as key to performance on the highest level in equestrian sport. The aim of this study was to quantify the extent of specialization and to evaluate its impact on analyses of genetic trends in sport horses in Germany. Information on the numbers of progeny with competition records in dressage and show jumping, available for 5,254 approved sires, was used to distinguish between specialized (at least 70 % of all competing progeny in either dressage, D, or show jumping, J) and allround stallions (A). This stallion classification was applied to sires and maternal grandsires of horses with genomic breeding values from the new routine single-step genomic evaluation for linear conformation, movement and jumping traits. This system uses results of linear descriptions from all major studbooks of riding horses in Germany (phenotypes from 2012 to 2025; > 81,000 horses) together with the genome-wide SNP genotypic data made available for their breeding programs (mostly 80K / Equine80select array; > 131,000 horses) plus pedigree information of four ancestral generations. For further analyses, genomic breeding values (gEBV) for all 28 conformation, 19 movement and 12 jumping traits of 315,266 horses born 1985 and later were considered. Stallions category data for sire and maternal grandsire was complete for 196,201 of these horses and indicated an increasing proportion of horses descending from D sire and D maternal grandsire or J sire and J maternal grandsire (> 80 % for birth years 2010 and later). More specialized training and competing in recent than former years has likely contributed to the observed relative decrease of horses classified as A (A sire and A maternal grandsire or combination of different categories). Genetic trends were determined by calculating of mean gEBV per birth year for either all horses (N = 315,266) or separately for D horses (N = 68,527) and J horses (N = 77,801). Results for overall genetic trends revealed mostly minor changes for conformation traits, whereas increases in performance-related traits were more obvious for movement than for jumping (increases of mean gEBV by around 15 vs. 5 points since 1985). Considering specialization, we found obvious discipline-specific patterns of genetic trends in the specialized horses: strongly positive trend for movement (+ 25 points or more) and negative trend for jumping in D horses, slightly positive trend for movement and strongly positive trend for jumping (up to +20 points) in J horses. Genetic trends for some of the conformation traits indicated differences in development in relation to discipline-specialization. This study has illustrated that analyses of genetic trends for the whole sport horse population or within a non-specialized studbook may lead to underestimation in genetic progress in the discipline-specific target traits and involves the risk of missing possibly unfavorable developments in functionality aspects.

Parentage Verification in Pura Raza Española Horses: Transition from Microsatellites to SNP-Based Genotyping Using the EQUIGENE 90K Array

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Maintaining studbook integrity through accurate parentage verification is fundamental to horse breeding programs. The Pura Raza Española (PRE), with over 290,000 registered horses worldwide, has relied on microsatellite-based parentage testing for over 20 years. However, the integration of genomic selection requires SNP genotyping, creating an opportunity to transition parentage verification from microsatellites to SNP-based platforms. ANCCE developed the custom EQUIGENE 90K array incorporating 90,926 SNPs, including the complete ISAG recommended parentage panel (859 SNPs), to serve multiple purposes: disease detection, evaluation of economically important traits, performance characteristics, and genomic selection. The objectives were to validate SNP-based parentage verification accuracy in challenging scenarios with closely related candidate parents, and to implement imputation from ANCCE's 19-microsatellite panel to facilitate connection of newborn foals with their parents using historical parentage records. Parentage verification was validated using 1,235 parent-offspring trios, including animals with close familial relationships. The ISAG compatible SNP panel demonstrated assignment accuracy exceeding 99.5% . The inclusion of challenging scenarios with closely related candidate parents demonstrated the superior power of SNP-based verification. In cases where full siblings exhibited up to 98.6% SNP compatibility and half-siblings showed 94.2–97.8% compatibility, the SNP panel maintained superior discrimination power versus traditional microsatellite approaches. These challenging scenarios provided evidence that when dealing with consanguinity, maintaining stringent thresholds near 1% incompatibility is essential for reliable parentage assignment. To enable integration of over 20 years of historical parentage records and facilitate verification of newborn foals using parents previously genotyped with microsatellites, imputation from the 19-microsatellite panel to equivalent SNP markers was implemented. This imputation strategy allows the connection of new generations genotyped with the EQUIGENE array to historical pedigree data, maintaining pedigree continuity during the transition from microsatellite to SNP-based platforms. In conclusion, the EQUIGENE 90K array successfully integrates the ISAG parentage panel, providing highly accurate verification that surpasses traditional methods, particularly in challenging scenarios with closely related individuals. The implementation of microsatellite-to-SNP imputation enables integration of over 20 years of verified parentage data, allowing connection of newborn foals to parents in the historical database.

Population structure and inbreeding in the Old Kladruber horse

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The Old Kladruber horse is a unique Czech autochthonous breed with pedigree records dating back to the eighteenth century. This population is closed, with a limited number of individuals, and is included in genetic resources. Therefore, it requires continuous monitoring of genetic diversity. In this context, the availability of long-term, well-maintained breeding records represents a considerable advantage. The aim of this study was to assess genetic diversity using genealogical and molecular (SNPs) data. For this purpose, a total of 9,940 pedigree records of animals born between 1729 and 2023 were utilized. The reference population comprised active animals (stallions and mares) born between 2000 and 2023 (n = 516). Pedigree analyses were performed using CFC and Retriever software. At least 98 % of animals in the reference population had five or more generations fully documented, with the average equivalent complete generations ranging from 10.73 to 12.66. The top 10 ancestors were identified, and the first four most important ancestors accounted for 31 % of the total genetic variability in the reference population. The average generation interval was long, at 12.43 years. All individuals included in the reference population were inbred. The average inbreeding coefficient showed a gradual, continuous year-to-year increase, reaching a maximum of 12.05 % in animals born in 2017. The average inbreeding rate was 1.11 %, and the corresponding effective population size equals 45. These parameters are slightly above (ΔF), respectively below (Ne) the FAO-recommended limits for the conservation of genetic diversity. These findings were supported by a genomic analysis of 420 individuals using 53,420 SNPs, which revealed an average genomic inbreeding coefficient of 14 % and an effective population size of 53 individuals. Overall, the results provide important insights into the current level of genetic diversity of the Old Kladruber horse and may support future conservation and breeding strategies for this unique breed. This work was supported by the GACR 24-14325L project.

Interactions between microplastics and the gastrointestinal ecosystem of livestock: integrating experimental evidence and emerging concepts

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Microplastics (MP) have recently emerged as a potential contaminant in livestock production systems through feed, water, and environmental exposure. Despite increasing attention in environmental sciences, the biological relevance of MP for gastrointestinal physiology and microbiology in livestock remains poorly understood. The aim of this presentation is to integrate current conceptual understanding of MP–host interactions in the gastrointestinal tract with recent experimental findings from controlled gastrointestinal simulation. Recent in vitro experiments employing rumen simulation systems assessed the effects of various MP types, which were combinations of different polymer species (polylactide, polyhydroxybutyrate, high-density polyethylene (HDPE), polyvinyl chloride, polyproylene), particle-size classes (<125 µm; 125-500 µm), and dosages (0-70 mg/incubation cylinder) of MP on ruminal fermentation and microbial function. Across all MP types overall fermentation performance decreased, and metaproteomic analyses indicated a functional shift towards stress and repair responses within the microbial ecosystem accompanied with declines in metabolic categories linked to carbohydrate and amino acid metabolism. In parallel, ruminal dry matter disappearance increased dose-dependently over the range of MP inclusion, which is consistent with interactions of MP with ruminal degradation processes and suggests that even chemically inert polymers such as high-density polyethylene (HDPE) may undergo measurable alteration under ruminal conditions. Collectively, these findings support the hypothesis that MPs may act less as classical toxicants and more as physical and chemical interfaces within the digestive ecosystem. A broader synthesis of the emerging literature indicates that the gastrointestinal tract represents a complex interaction network where MPs may influence microbial communities, digestive processes, and host–microbe interactions through several mechanisms. Proposed pathways include particle–microbe attachment processes, adsorption and release of organic compounds on polymer surfaces, and physical interactions with mucus and epithelial barriers. In livestock, these interactions may be further modified by the highly dynamic physicochemical environment of digestive compartments such as the rumen. Taken together, current evidence suggests that the biological significance of MPs in livestock is unlikely to be explained solely by toxicological paradigms derived from aquatic models. Instead, a systems-oriented perspective focusing on digestive microbiology and host–microbe interactions appears more appropriate. Future research should therefore combine controlled experimental approaches with microbiome and functional analyses to better understand whether MP exposure can affect digestive ecosystem stability, nutrient utilization, or animal health. This overview highlights how integrating experimental evidence with conceptual frameworks can help refine hypotheses and guide future research on MPs in livestock production systems.

Interaction of polyethylene terephthalate microplastics within the rumen environment

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Microplastics (MPs) are increasingly detected in livestock feeds, drinking water, animal tissues, feces, and animal products, raising concerns about their potential interaction with ruminal physiology. The rumen represents a primary site where MPs may simultaneously affect nutrient degradation and undergo transformation. This study aimed to evaluate the effects of polyethylene terephthalate (PET) MPs on ruminal degradability and fermentation parameters of different feed matrices (mixed hay, concentrate, and total mixed ration [TMR]), and to determine the extent of PET MPs degradation under simulated ruminal conditions. Mixed hay degradability and digestibility were evaluated using a three-step ruminal–gastrointestinal method (Ankom DaisyII incubator). PET MPs were added at 0, 5, 10, and 15 g/L of inoculum. Three different hays were tested in triplicate across three experimental runs (n = 108). PET MPs significantly reduced crude protein degradability in the ruminal phase (−9% and −16% at medium and highest doses, respectively) and decreased neutral detergent fiber degradability by up to 13%. In the intestinal phase, crude protein digestibility of rumen-undegraded residues decreased by 8% at the lowest PET level, indicating impaired protein and fiber utilization in forage-based diets. In a batch rumen fermentation model, concentrate feed degradability and fermentation parameters were evaluated with PET MPs included at 0, 0.6, 1.2, and 1.8% of feed dry matter (96 h incubation; n = 36). Results showed that PET MPs altered fermentation kinetics by increasing asymptotic gas production (6%) and average fermentation rate (13%), while shortening lag time (from 0.77 h to 0.26 h at the highest level). Despite increased gas output, dry matter and organic matter degradability decreased (−4.1% and −4.6%, respectively). Ammonia-nitrogen concentration increased (+6.7%), rumen protozoa declined linearly, and microbial efficiency was reduced, indicating disrupted fermentation balance and a potential decoupling between gas production and effective substrate degradation. TMR degradability was evaluated in the Ankom DaisyII incubator after 48 h of incubation with PET MPs of three particle-size fractions (25–150, 150–300, and >300 µm). The experiment included replicated treatments across multiple runs (n = 192). PET MPs reduced dry matter degradability (−1.9%) and fiber fractions degradability; however, the inhibitory effect was not particle-size dependent. In vitro ruminal degradation of PET MPs was evaluated using the Ankom DaisyII incubator with TMR as substrate. PET MP degradability was quantified gravimetrically as percentage weight loss. In the time-course experiment (24, 48, and 72 h; n = 54), PET exhibited progressive and significant degradation (0.50 ± 0.070%, 0.73 ± 0.057%, and 0.96 ± 0.082%, respectively; $p < 0.0001$). In a second experiment evaluating PET MPs of different particle sizes (25–150 µm, 150–300 µm, and >300 µm; n = 90), degradation after 48 h was particle-size dependent: small and medium particles reached values up to 1.50%, whereas larger particles showed lower weight loss (0.51%). Overall, PET MP degradation remained below 1.5% within typical ruminal retention times, indicating limited but measurable ruminal biodegradation, particularly for smaller particles. In conclusion, PET MPs negatively affect nutrient degradability in forage, concentrate, and TMR diets, while undergoing minimal degradation in the rumen environment.

Growth, jejunal integrity, and physiology of pigs are maintained with diets containing microplastics

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Microplastics (MP) are ubiquitous pollutants that have been found also in feed and feed ingredients for livestock, potentially affecting animal health and physiology. Alternative feed ingredients like former food products (FFPs), were selected to study this subject in a realistic scenario as the presence of MPs in FFPs has been reported while still within legal allowance. Two FFPs (analyzed in triplicate, 20 g each) were analyzed using microscopy and Fourier-transform infrared spectroscopy to identify plastic packaging fragments. Eight plastic fragments (average size = 6.7 mm) were detected in FFP1 and two (average size = 3.4 mm) in FFP2. Given these findings and the potential for macro- and mesoplastics to further break down into MPs during digestion, an in vivo trial was conducted using pigs as a model to examine the effects of feed-derived MPs on animal performance, apparent total tract digestibility (ATTD), and gut health. In total, 36 Swiss Large White male castrated pigs (22.38 ± 1.70 kg body weight) were assigned to one of the three growing and finishing diets (1) a standard diet containing no FFPs (ST); (2) a diet in which 30% of conventional feedstuffs were replaced by FFP1; and (3) a diet in which 30% of conventional feedstuffs were replaced by FFP2. For each pig, average feed intake, average daily gain, feed efficiency and ATTD were monitored. A mass spectrometry-based untargeted metabolomics investigation was performed on plasma samples from all the animals. Twenty-four fecal samples were collected from the rectal ampulla to analyze fecal volatile fatty acids level and microbiota population using high-performance liquid chromatography and next-generation sequencing, respectively. Fecal MP content was also quantified. Organic matter was digested with 30% H2O2, and a saturated NaCl solution (D = 1.2 g/cm3) was added to separate target MPs (polyethylene and polypropylene) into the supernatant. Two jejunal samples from each pig (≥ 7 pigs per treatment) were collected and mounted in Ussing chambers to evaluate the trans-epithelial resistance (TEER) to assess jejunal epithelial integrity. All data were analyzed by analysis of variance using linear mixed-effects regression models in R v4.0.3, except that the packages phyloseq, vegan, microbiome, and microbiomeutilities were used for microbiota data analysis. Both FFPs decreased fiber ATTD in the finishing phase but FFP1 increased the energy and crude protein ATTD in the growing period. The type of FFPs and the related MP contamination level did not affect ($P > 0.05$) pig's growth traits, plasma metabolome, TEER, and fecal microbiota abundance or biodiversity (Shannon index) and fecal volatile fatty production. No MP was observed in the fecal samples by using the selected method. This indicates that the contamination level of plastics in FFPs, does not cause immediate observable negative effects on pig's performance and gut health. Further studies with controlled feed spiking at different MP concentration are warranted to investigate whether feed-sourced MPs can remain in carcass and meat and alter their quality. Additionally, other more accurate MP extraction methods for complex organic matrices like feces has to be tested.

Analysis of microplastics in black soldier fly larvae exposed to packaging materials

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Reared insects are increasingly seen as an alternative protein source. Black soldier fly larvae (BSFL, Hermetia illucens (L.) Diperera: Stratiomyidae) have a high potential for circular use in the feed chain, because they can be reared on a variety of materials unsuitable for 'conventional' livestock. One such material includes former foodstuffs (FFS) with elevated levels of packaging materials. At this time, remnants of packaging are prohibited in feed, but complete removal not feasible. We hypothesized that BSFL would not be affected by the presence of packaging materials in feed, and that the resulting larval biomass would be safe as a protein source for feeding to other animals. The aims of this project are to gather more data on the safety of rearing BSFL on feed with (biodegradable) plastics, to enable risk assessment by the competent authorities. To properly assess the possible hazards arising from rearing insects on FFS containing packaging materials, physical (microplastics) and chemical (contaminants) hazards are researched. Effects of these materials on the assayed insects (e.g., yield and survival) will also be monitored, as well as possible (bio)degradation of the plastics by the insects. Included in this presentation are the first results from microscopy-based analyses of BSFL that had been exposed to different forms of plastic packaging materials in the substrate. In order to detect possible (micro)plastics in the larvae, a tailored digestion method was employed to remove organic biomass while not affecting the plastic integrity. Scanning microscopy allowed to generate large-scale images of slides, using polarization-based optical filtering to contrast remaining biomass with the microplastics.

Transcriptomic profiling of aflatoxin B1 exposed and medicinal herb supplemented pig liver

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Aflatoxin B1 (AFB1) is a toxic secondary metabolite produced by Aspergillus fungi. Dietary exposure of AFB1 strongly induces adverse effects in farm animals. EU feed legislation sets maximum AFB1 at 20 µg/kg in pig compound feed (piglets: 5 µg/kg; 12% moisture). With rising climate-driven contamination risk and non-exhaustive monitoring, especially for on-farm feed, contaminated lots can still enter rations and exceed legal limits. Chronic exposure of AFB1 in feed can lead to critical physiological and pathological consequences in animals, including reduced growth rates, reproductive issues, immunosuppression, and gut barrier dysfunctions. Since the complete eradication of AFB1 from feedstock through physical and chemical methods is ineffective, several natural additives with anti-toxic properties have been widely investigated as potential AFB1 mitigation strategies. Extracts from medicinal herbs and their constituent bioactive compounds have demonstrated significant hepatoprotective activity, aiding in the restoration of liver integrity. Accordingly, a nutritional experiment was performed incorporating AFB1 (120 µg/kg BW) exposure via feed; kalmegh (Andrographis paniculata: 30 mg/kg BW), milk thistle (Silybum marianum: 90 mg/kg BW) and turmeric (Curcuma longa: 90 mg/kg BW) supplementation (BW: body weight) in distinct pig groups, followed by RNA sequencing of liver tissue (n = 44). Serum biochemical parameters associated with liver health were analyzed to compliment the gene expression data. The analysis of differentially expressed genes (DEG) in AFB1-exposed animals revealed transcriptomic modulations in genes like CYP2A19, CYP1A2, GSS, GPT2, and PCK1 related to AFB1 primary biotransformation, mounting of toxic responses and reduced energy derivation. Alternatively, with medicinal herbs, a positive adaptive regulation of respective genes CYP27A1, ACSS2, and HAMP was observed in terms of metabolic activation, energy and iron homeostasis. Furthermore, AFB1 exposure in pigs resulted in modulated serum hepatic parameters suggesting an onset of aflatoxicosis. Conversely, in medicinal herb supplemented animals, the absence of significant differences in biochemical parameters levels indicated an unaffected healthy liver function. These findings provide a foundation for delineating the specific molecular mechanisms related to both AFB1 exposure and medicinal herb supplementation and account for possible nutritional strategies in future aimed at mitigating AFB1 toxicity in animals using natural additives. This project has received funding from the Deutsche Forschungsgemeinschaft (DFG) Project No. 504983453 (WI 1754/18) and Narodowe Centrum Nauki (NCN) Project No. UMO-2021/43/I/NZ9/02612.

Microplastics in animal-based food products: occurrence, contamination pathways, and analytical challenges

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Microplastics are plastic particles smaller than 5 mm, generated either through the intentional production of small-size plastics or through the fragmentation of larger plastic items. Their persistence and widespread environmental distribution have raised increasing concern regarding their entry into the food chain, including livestock production systems. While marine ecosystems have been extensively investigated, there is still a lack of studies investigating the occurrence of microplastics in animal-based food products. The aim of this contribution is to provide a comprehensive overview of current knowledge on microplastic contamination in animal-based foods, linking environmental exposure, livestock management practices, and food processing dynamics along the production chain. In farm animals, ingestion through contaminated feed, water, soil, and airborne particles represents the primary exposure route. Although the extent of biological transfer from feed to animal tissues remains under investigation, organs involved in product formation, such as the mammary gland and reproductive system, represent potential interfaces for particle translocation. In addition, contamination may occur during milking, slaughtering, processing, packaging and storage, reflecting the complexity of modern production systems. To date, major uncertainties persist regarding analytical harmonization, particle characterization, contamination sources, and quantitative exposure assessment. In particular, the lack of standardized analytical protocols hampers by far cross-study interpretation. Notably, specific regulatory thresholds for microplastics in food are currently lacking, and risk assessment frameworks remain constrained by limited clinical data at both animal and human level. By integrating evidence from different animal-based matrices and identifying critical points along the production chain, this work highlights priority research needs and methodological challenges. Strengthening harmonized analytical approaches and refining exposure assessment models will be essential to support the development of science-based regulation framework and mitigation strategies aimed at reducing microplastics diffusion and circulation.

LC–MS/MS determination of pesticide and antibiotic residues in milk: effects of thermal processing and composition

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In pasture-based dairy systems, cattle may be exposed to chemical residues from both environmental sources, such as pesticides used in crop production, and the use of veterinary drugs, posing food safety concerns. Dairy processing treatments such as pasteurization and ultra-high temperature (UHT) may influence residue stability, distribution and concentration within the milk matrix, making their behavior relevant for risk assessment and regulatory monitoring. This study investigated whether thermal processing modifies the fate of pesticide and antibiotic residues through compound-specific degradation and partitioning within the milk matrix. A multi-residue LC–MS/MS method was developed and validated to assess the impact of thermal processing and milk composition on pesticide and antibiotic residues in milk following SANTE/11312/2021 guidelines. A solid–liquid extraction procedure was optimized for the determination of selected pesticides and veterinary drugs relevant to the Uruguayan production context using LC–MS/MS. Method validation included evaluation of trueness (% recovery), precision (% RSD), selectivity, limits of quantification, linearity, robustness and matrix effects. Acceptance criteria were recoveries between 70–120 %, RSD ≤20 % and linearity with back-calculated concentrations ≤20 %. To evaluate residue behavior during dairy processing, raw milk previously confirmed as residue-free was fortified with selected pesticides and veterinary drugs at 50 µg/kg and subjected to thermal treatments in a laboratory-scale fluid milk processing line. Thermal treatments included pasteurization (72 °C for 15 s; 3 L/h) and UHT processing (142 °C for 4 s; 20 L/h). Representative samples (n = 3) were collected at different stages of processing to monitor changes in residue concentrations. Data were analyzed using analysis of variance (ANOVA) followed by Tukey's test ($p < 0.05$). Results showed the relevance of the milk lipid fraction on contaminant distribution. Highly lipophilic compounds such as abamectin, carbaryl, diazinon and fluazuron showed a clear tendency to partition into the cream fraction, reaching concentrations of 40–210 µg/kg, whereas considerably lower levels were detected in skim milk (2–49 µg/kg). In contrast, other analytes exhibited a more homogeneous distribution between lipid and aqueous phases. For both thermal treatments, residue concentrations of compounds such as fluazuron and fipronil remained stable ($p > 0.05$), consistent with previous reports by Rose et al. (2017). Additionally, milk standardization at different fat contents (2.6% and 3.0%) resulted in a greater decrease in overall contaminant concentrations in samples with lower fat content. These findings demonstrate that milk composition plays a key role in determining the fate of pesticide and antibiotic residues during dairy processing. The proposed analytical approach provides a robust tool for studying contaminant behavior in dairy systems and contributes to improving residue monitoring and food safety management in dairy production chains.

Replacement of hexane-defatted oilseed meals with 2-methyloxolane-defatted oilseed meals in dairy cows' diet: Effects on dairy performance and nitrogen utilisation

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Hexane (HEX) is used to defat oilseed meals fed to livestock. However, HEX is sourced from petroleum and its main compound, n-hexane, is considered a substance of very high concern in the European Union. A biobased solvent, 2-methyloxolane (MeOx), was recently authorised for food extraction and could be an alternative to HEX. Two previous studies investigated the use of MeOx-defatted soybean meal (SBM) in the diets of dairy cows and growing beef cattle. However, only SBM was studied, and it contained a high level of MeOx residues (977 mg of MeOx/kg DM).Our objective was to evaluate the effects of HEX- vs. MeOx-defatted SBM and rapeseed meal (RSM) on dairy cows' performance and N utilisation.Thirty-six dairy cows (10 primiparous and 10 multiparous Holstein, 10 primiparous and 6 multiparous Montbéliarde) were enrolled in a continuous factorial experimental design with 2 oilseeds (SBM and RSM) × 2 solvents (HEX and MeOx): SBMHEX, SBMMeOx, RSMHEX, and RSMMeOx. The experiment lasted 9 weeks (4-week pre-experimental phase with a common diet + 5-week experimental phase with the experimental diets). The experimental diets were iso-crude protein (CP, 140 g/kg DM) and consisted mainly of hays, a mixture of maize, wheat, and barley grains, and one of the experimental oilseed meals providing 36% of the CP intake. Individual feed intake, milk yield, milk protein and fat contents, and N utilisation traits (ruminal fluid NH3, milk urea, plasma urea and amino acids [AA], N partitioning) were measured in week 9. A fixed linear model including oilseed, solvent, oilseed × solvent, breed, and parity was built. When a covariate was also measured in week 3, the breed and parity effects were replaced by the covariate effect. Data were analysed using equivalence tests.Oilseed meals defatted with MeOx contained 29 to 53 mg/kg DM of MeOx residues, and oilseed meals defatted with HEX contained 42 to 47 g/kg DM of n-hexane residues. Milk yield, milk protein and fat contents, and N partitioning were likely equivalent between SBMHEX and SBMMeOx, despite DM intake (DMI) being unlikely equivalent toward higher intake for SBMMeOx(+1.6 kg/d). With RSM, the DMI and milk protein and fat contents were likely equivalent between RSMHEX and RSMMeOx, but milk yield and N milk:N intake were unlikely equivalent toward lower values for RSMMeOx (−2.2 kg/d and −1.9 g/100 g). Ruminal fluid NH3 and milk urea were unlikely equivalents toward lower values for MeOx compared with HEX for both SBM (−32.4 and −26 mg/L) and RSM (−21.8 and −30 mg/L). Plasma total essential AA (EAA) and urea were likely equivalent between SBMHEX and SBMMeOx, but plasma lysine was unlikely equivalent toward higher concentrations for SBMMeOx (+5.9 µmol/L). Plasma total EAA, lysine, and urea were unlikely equivalent toward lower concentrations for RSMMeOx compared with RSMHEX (−153 µmol/L, −20 µmol/L, and −0.06 g/L).Results on ruminal NH3 and milk urea, as well as on plasma urea for RSM, suggest an unlikely equivalent ruminal CP degradability of properly desolventised oilseed meals, toward lower degradability for MeOx compared with HEX. Future studies may investigate the intestinal digestibility of rumen undegradable protein from properly desolventised MeOx-defatted oilseed meals.

Voluntary feed intake, body composition and metabolic status differed between growing beef cattle fed hexane vs bio-based solvent-extracted soybean meal in a 7-months feeding trial

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The use of hexane (HEX) for soybean meal (SBM) production is under discussion because of safety concerns. The 2-methyloxolane (MeOx) is an alternative, bio-based solvent, that requires long-term evaluation regarding animal performance and metabolic effects of chronic exposure. This study evaluated the use of HEX- vs MeOx-extracted SBM, supplemented (7 g/d of Smartamine®) or not with rumen protected methionine, on the performance and metabolism of 36 growing-fattening Charolais young bulls [251 ± 22 days of age, 337 ± 39 kg body weight, (BW), n = 9/treatment] across three consecutive feed efficiency test periods (70 days each). All diets had a similar feed composition (main composition: grass silage 55 %, energy concentrates 29.4 %, SBM 9 %, DM basis). Diets contained 1.0 mg HEX/kg DM and 89.8 mg MeOx /kg DM, respectively. The dry matter intake (DMI, daily) and BW (fortnightly) were recorded and the average daily gain (ADG) and feed conversion efficiency (FCE) were calculated for each period. Blood plasma samples were taken at the midpoint of each and stored (−80 °C) until the analysis of non-esterified fatty acids (NEFA), glucose, insulin and leptin through commercial colorimetric or ELISA kits. The surrogate insulin sensitivity indexes RQUICKI and HOMA were calculated. The plasma metabolomic profile was determined only in period 1 (MxP Quant 500 kit, Biocrates Life Sciences, Austria). At slaughter, carcass composition was estimated based on the 6th rib composition. Data were analysed as repeated measures (fixed effects: SBM type, Met supplementation, the period and all their interactions; random effect: the animal; covariance structure: AR(1)) in SAS 9.4 (SAS Institute Inc., Cary, NC, USA). Metabolomic data, and carcass traits at slaughter were analysed considering the SBM, Met and their interaction as fixed effects. Tukey test was used for post-hoc comparisons. Differences were declared at $P \leq 0.05$ and tendencies at $P \leq 0.10$. False discovery rate (FDR)-adjusted P-values were also applied for metabolomics data. The DMI was higher in MeOx vs HEX only in period 3 (SM×Period $P<0.01$). The FCE, ADG and carcass weight increased with Met supplementation only in the MeOx diets ($P \leq 0.05$). Fat content of the 6th rib was higher in HEX vs MeOx regardless of Met ($P=0.05$); no evidence of difference was found for muscle and bone tissues contents. Estimated carcass fat (+3.6 %), muscle (−0.9%), and muscle-to-fat ratio (−4.3 %) tended to differ in HEX vs MeOx diets regardless of the Met ($P \leq 0.08$). Plasma NEFA increased ($P<0.01$) from period 1 to 2 only in MeOx (SM×Period $P=0.04$). Regardless of the Met level, plasma glucose tended to be higher ($P=0.07$) in HEX vs MeOx only in period 3 (SM×Period $P=0.09$). Plasma insulin was overall higher in HEX vs MeOx ($P=0.01$) regardless of Met; leptin was higher in HEX vs MeOx only when Met was supplemented (SM×Met $P=0.10$). The HOMA index was overall higher in HEX vs MeOx, and RQUICKI was higher in HEX vs MeOx only in period 1 (SM×Period $P=0.07$). Several long-chain poly-unsaturated phosphatidylcholines were lower in HEX vs MeOx ($P<0.05$, FDR>0.10). Our results suggest that chronic exposure to hexane may cause subclinical yet evident alterations in energy and lipid metabolism of growing beef cattle, potentially impacting the long-term control of voluntary feed intake. This study may provide the first experimental indication of adverse metabolic effects of chronic dietary exposure to low levels of hexane residues in mammals.

Determining the impact of anaerobic digestion on the host-range and potential mobility of antimicrobial resistance genes in dairy manure

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Antimicrobial resistance (AMR) is a critical One Health threat, with livestock manure serving as a major environmental reservoir of antimicrobial resistance genes (ARGs) and mobile genetic elements (MGEs). Reducing resistance dissemination at the manure management stage represents a strategic intervention point. Anaerobic digestion (AD), widely used for organic waste stabilization and biogas production, has been proposed as an effective strategy to reduce pathogen and ARG loads in manure. However, its impact on the dynamics of ARG carrying microbiome (resistome) and associated MGEs (mobilome) remains poorly understood. In this study, we investigated temporal shifts in microbial community composition, ARG abundance, and ARG–MGE associations during laboratory-scale AD of dairy manure. Manure from a commercial dairy farm was fed into a continuously stirred AD at 3 one-month intervals. Samples were collected from manure, 0 and 1 h post feeding the AD, and 1, 2, 3, and 4 wks post-feeding. Samples were processed through two pipelines: direct shotgun metagenomics using Illumina short-read sequencing, and culture-enriched metagenomics (CEMG) under aerobic and anaerobic conditions using a hybrid metagenomic approach (Illumina and Nanopore sequencing). For CEMG, samples were plated on brain heart infusion agar supplemented individually with ceftiofur, lincomycin, tetracycline, vancomycin, erythromycin, or ampicillin, each applied at their respective recommended breakpoint concentrations, to enrich low-abundance taxa associated resistomes. Enriched cultures were pooled and sequenced using Illumina and Oxford Nanopore platforms, to enable hybrid metagenomic assembly and generation of a high-quality contig database. A total of 196 contigs exhibited ARG–MGE co-localization. Direct metagenomic reads from all time points were mapped to this database to monitor temporal shifts in ARG-carrying taxa and mobility linkages. AD induced rapid and substantial ecological restructuring; Streptococcus parauberis and Vagococcus fluvialis, abundant in raw manure, were eliminated from digestate samples from 1 h through wk 4. At the order level, fresh digestate exhibited a 83–95% reduction in dominant manure-associated taxa, including Bacillales, Peptostreptococcales, Eggerthellales, and Pseudomonadales. Although Bacteroidales displayed transient enrichment at wk 2 (+30.6%), its abundance remained 30.7% lower than manure by wk 4. Lactobacillales fluctuated during AD, but declined by 82% at the final time point. Total ARG content declined sharply following AD, with fresh digestate showing an 83% reduction relative to manure and a maximum reduction of 89% at wk 2. Despite a modest increase at wks 3 and 4, ARG levels remained 4-9-fold lower than untreated manure. ARGs conferring resistance to clinically relevant drug classes, including aminoglycosides, lincosamides, and tetracyclines, exhibited sustained suppression during AD, remaining approximately 80-97% lower than raw manure by wk 4. Notably, these ARGs were linked to diverse MGEs, including plasmids, integrative genetic elements (IGEs), and phages, highlighting a concurrent reduction in mobilizable resistance potential. Overall MGE abundance declined by 40-45% across the digestion timeline. Collectively, AD induced marked ecological restructuring and substantial resistome attenuation in livestock manure, reducing ARG burden while diminishing mobility potential.

Utilizing the shape of the heart to decrease mortality in pig breeding.

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Heart failure is a known cause of sudden death in pigs, but the underlying morphological and clinical factors, such as cardiac remodeling and reduced stroke volume are difficult to assess non-invasively in live animals. The external surface of the heart can be extracted from full-body CT images, and previous studies have shown an association between large heart volumes and increased mortality. However, direct selection for smaller heart size may not be a sustainable long-term strategy. In this study, we examine whether the shape of the pig heart surface contains additional biologically relevant information that could support breeding programs beyond what volume alone provides. Using image registration techniques on 1,849 segmented hearts from a synthetic line, we extracted 481 surface coordinates to create a structured multivariate dataset. Principal component analysis (PCA) showed that the first three components explained 70% of the variation in heart shape. We then applied partial least squares (PLS) regression to identify shape features correlated with relevant response variables, such as estimated breeding values (EBVs) for mortality from rearing to finishing. Surface coordinates and corresponding EBVs were split into an 80% training set and 20% test set. The highest predictive performance was obtained with two PLS components, yielding an R^2 of 0.032 for the test set. Using the full dataset with two components produced a similar R^2 of 0.033, corresponding to a correlation of 0.18. Both PLS score vectors were correlated with heart volume (r = 0.80 and 0.32). These findings indicate that limited additional information related to mortality exists in heart surface shape beyond total heart volume. Heart volume itself showed a correlation of 0.13 with mortality EBV, confirming its importance. To exploit CT-derived cardiac phenotypes for improving survival in pig breeding, we propose incorporating heart volume as a correlated trait within multivariate EBV prediction models.

Milking-time indicators link vacuum dynamics, milk flow, and milk quality in Holstein herds

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Variations in milking vacuum affect mechanical stress on teat tissue, with potential consequences for udder health and milk quality. This study evaluated key milking-time test indicators in commercial Holstein herds, and their contemporaneous associations with milk yield, composition, and somatic cell score (SCS). Data comprised 4,707 test-day records from 4,149 cows in 215 Italian herds equipped with conventional milking parlors. Vacuum was continuously recorded at the short milk tube (SMT) and mouthpiece chamber (MPC) to characterize machine-on time, milk let-down (MLD), overmilking, positive and negative cyclic vacuum fluctuations (CVFmax and CVFmin, respectively). A random forest model identified the milking-time test, herd and animal indicators most strongly associated with variation in SCS. Therefore, the most relevant milking-time test indicators were included in repeated linear mixed models to quantify their effects on milk yield and composition. Moreover, similar mixed model approaches were used to determine how the most important herd and animal characteristics influenced milking-time test indicators. Overmilking duration, SMT vacuum and positive and negative CVFs during the main milking phase were the variables most strongly associated with SCS variation. SCS significantly increased when overmilking exceeded 30 s and peaked beyond 60 s (2.58 vs. 2.93, respectively), while lactose was significantly lower in the ≥60 s class (4.78%), milk yield per session was unaffected. SMT vacuum influenced milk yield and composition: SCS were significantly highest at 37.3-38.0 kPa (3.08) and lowest at 39.3-39.8 kPa (2.45), while lactose was lowest between 36.7-37.3 kPa (4.76%) and greatest between 38.7 and 39.8 kPa. Greater CVFmin and CVFmax were significantly associated with reduced fat content once fluctuations exceeded ~ 3.50 kPa. Large-size herds (>329 lactating cows) had significantly shorter machine-on time (323 vs. 337 s) and overmilking (47.7 vs. 59.3 s), higher milk flow (3.11 vs. 2.70 kg/min), consistent with the higher-genetics cow populations which can likely be found in larger operations. However, large size herds showed significantly longer milk let-down duration (34.8 vs. 24.7 s), likely reflecting reduced pre-stimulation in high-throughput routines. Similar trends were observed in high-yielding herds (>38 kg/day) that had significant shorter machine-on time (326 vs. 340 s), lower relative overmilking (15.1% vs. 18.4%), longer milk let-down (9.67% vs. 8.50%), and higher milk flow (3.17 vs. 2.73 kg/min) compared with low-yielding herds (<34 kg/day). Finally, MPC vacuum significantly declined as parity order increased, from 21.8 kPa in primiparous cows to 12.8 kPa in cows ≥4 parities, consistent with progressive teat canal widening. Overall, milking-time test indicators captured biologically relevant variation in milk yield and composition. Their integration into routine milking assessments may support early detection of deviations and targeted adjustments of milking protocols to improve udder health and efficiency.

A machine learning framework for early and dynamic prediction of milk, fat, and protein yield of dairy cows

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Accurate prediction of lactation curves for milk, fat, and protein yield of individual cows is essential for decision-making in farms and dairy processing. They serve as basis for herd planning and insemination decisions, cow-comparison key figures such as net returns and lactation index, and forecasting in dairy plant production planning. By comparing the expected production with the actual yields, the model enables early detection of deviations that may signal health or welfare concerns, supporting proactive interventions. This study presents a machine learning–based lactation model capable of predicting daily milk, fat, and protein yields for individual cows up to 500 days in milk, both prior to calving and dynamically throughout lactation after new test-day records become available. The model incorporates genetic information, herd levels, animal levels, pregnancy status, and auxiliary characteristics such as parity, calving season, and age. The herd and animal level effects express herd and animal performance relative to population standard curves. It is trained and tested using a large dataset of test-day records from 1.9 million lactations from 8,981 Dutch herds, representing a highly diverse operational environment. This scale allows the model to generalize across herd types and management conditions, which is crucial for a robust model. Before calving, the model predicts the first 305 days of lactation with median absolute errors (MEDAE) of 3.64 kg for milk, 0.16 kg for fat, and 0.13 kg for protein yield, corresponding to percentage errors of 15.3%, 15.3%, and 14.6%, respectively. Prediction accuracy for later lactation days is slightly lower, largely due to the lack of reliable pregnancy information prior to calving. Once the lactation begins and new test-day records are incorporated, accuracy improves, with the MEDAE decreasing to 2.45 kg milk, 0.13 kg fat, and 0.10 kg protein, and percentage errors of 11.3%, 12.5%, and 11.7%, respectively. The predictions provide a valuable benchmark for evaluating expected versus actual performance of the cow. As lactation progresses, incorporating new test-day data further refines accuracy, enabling reliable estimation of expected performance, dry-off yield, and cumulative (expected) 305-day and lactation production of an animal, herd, or herd groups, such as by parity. This information can be used as early-warning indicators of health and welfare concerns, as well as determining the optimal insemination moment which can extend the lactation. Extending lactations reduces the number of transition periods for the cow, which reduces the health risks and increases welfare. Because the model can forecast both ongoing and future lactations, it supports medium- to long-term planning and can be used to predict expected production for entire herds or herd groups up to two years ahead, contributing to healthier cows and improved decision-making.

Use of Thermography for Monitoring Udder Health Status in Dairy Cows

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Hypothesis. Precision livestock farming is currently seeking new alternative methods for monitoring animal health and welfare. Among these, infrared thermography is emerging as a technology that allows the acquisition and processing of thermal information, enabling the early detection of alterations in blood flow and skin temperature associated with various pathological processes. Thermography is a practical, reliable, and non-invasive method for monitoring animal health status. Today, it is mainly used in experimental studies on dairy cows to monitor hoof and udder health. It was hypothesized that udder surface temperature measured by infrared thermography is associated with mastitis infections, including subclinical cases, and with changes in milk production, milk flow parameters, and differential somatic cell count. Experimental methods. Data were collected weekly using a FLIR E96 thermal camera to capture thermal images of the udder approximately two hours before milking; milking was monitored using continuous electronic milk flow meters (LactoCorder, WMB, Balgach, Switzerland). Milk samples were collected to evaluate the differential somatic cell count and the concentration (cells/mL) of neutrophils, macrophages, and lymphocytes using the Vetscan DCQ Milk Analyzer. Statistical analyses. Data processing and analysis were carried out using Microsoft Excel and JMP software. Generalized Linear Mixed Model (GLMM) analyses were conducted to investigate the relationships among the different measured parameters. Results and discussion. The average milk yield during the study period was 14.0±4.09 kg per milking, with a maximum milk flow rate of 3.56±1.27 kg/min. Another interesting parameter evaluated was the percentage duration of the plateau phase, which averaged 34.8±20.87% of the total milking time. Regarding udder health variables, maximum electrical conductivity (6.00±0.47 mS/cm) and total leukocyte somatic cell count (TLC; 4.72±0.59 $\log_{10}$ cells/mL) were considered. In terms of average percentage composition, neutrophils represented the largest proportion (53.8± 13.3%), followed by macrophages (30.7±15.2%) and lymphocytes (15.6±8.10%). Concerning the temperature measured on the udder surface, both the mean and maximum values were evaluated for each cow; on average, these values were 35.1±0.90°C and 38.4±0.88°C, respectively. For TLC values above 5 $\log_{10}$ cells/mL, reductions of up to 5.4 kg of milk per milking and up to 14.4% in the percentage plateau phase were recorded; moreover, maximum electrical conductivity increased by 0.38 mS/cm were observed. These parameters may be considered early indicators of the presence of a mammary infection. The temperature measured on the udder surface did not show a significant relationship with parameters directly associated with udder health: despite increases in total leukocyte somatic cell count and electrical conductivity, maximum temperature remained stable at around 38.2°C. Instead, it appeared to be more closely associated with milk yield: when production exceeded 16 kg per milking, an increase in udder surface temperature of up to 0.6°C was observed. Conclusion. The importance of production parameters for monitoring, through the integrated use of various tools, udder health status and identifying possible diseases is emphasized; at the same time, thermography can be used to identify healthy cows with a more active and well-functioning metabolism.

Methods to describe the behavioural resilience of cows after a systemic inflammatory challenge

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Resilience is the capability of an animal to be minimally affected by a disturbance or to return rapidly to the state before the disturbance. A change in behaviour is an early indicator of disease and could indicate the capacity of the animal to cope with the disease. We aimed at designing a mathematical method to assess the behavioural resilience of cows to further study the links between behavioural and clinical resilience. Fourteen Holstein cows were challenged with intravenous lipopolysaccharide (LPS) injection. The behaviour of the cows was continuously monitored using collar-attached accelerometers from 30 days before to 60 days after LPS injection (T0). Theoretical points in time after T0 were defined: the beginning of the deviation of resting from baseline (Tb) and the end of LPS impact on resting behaviour (Te). Two mathematical methods were tested: a regression line method on the cumulative data (RC) and a regression line method on the first derivation of the cumulative data (RD). The baseline for resting behaviour was defined using a regression based or on the cumulative resting time per hour (RC) or on the first derivation of the cumulative resting time (RD) over the last six days before T0. In both methods, Tb was the first point exceeding the baseline ± 2 SD. Te was detected by RC when the regression line stayed within 5% of the baseline slope before T0, and when 95% of the residuals were within ±2 SD of the baseline, with both conditions satisfied for at least six consecutive days. Te was detected by RD when the values returned to the baseline ± 2 SD for at least six consecutive days. Four experts (biologists) identified Tb and Te by visual observations of the first derivation of the cumulative data. The points (Tb, Te) from RC and RD were compared with those of experts using Bland-Altman analysis (bias and 95% limits of agreement (LoA)) and calculating the Lin's concordance correlation coefficient (CCC). Both mathematical methods and experts found Tb in all 14 cows. Te was found in 10/14 cows using RC, 11/14 cows using RD and in 12/14 cows by the experts. Tb was found at 4.5 h (± 8.0) by RC, -7.2 h (± 6.7) by RD and -14.2 h (± 13.1) by experts. Te was found at 190.8 h (± 42.7) by RC, 155.9 h (± 58.1) by RD and 194.8 h (± 21.5) by experts. Between RC and experts, a bias of 19 h [lower LoA –upper LoA] [(-17) – 45 h] for Tb and a bias of -12 [(-112) – 88] for Te, and between RD and expert a bias of 7 [(-29) – 43] for Tb and a bias of -39 [(-170) – 92)] for Te. Between RC and experts, the CCC was 0.4 for Tb and 0.2 for Te. The CCC was 0.3 and 0.4 between RD and experts for Tb and Te, respectively. Both mathematical methods were able to identify both points of interest with a smaller marge of error for Tb than Te. For now, the RC method appears to produce results closer to those of the experts than the RD method when determining Tb. No clear distinction was observed between the two mathematical methods when the bias, LoA and CCC of both methods were considered for Te. Further work is ongoing to refine both mathematical methods and then apply them on clinical parameters in order to study the links between behavioural and clinical resilience.

Inferring transmission routes of Staphylococcus aureus using high-resolution contact data in dairy cattle

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Understanding how mastitis-causing bacteria spread within dairy herds is essential for designing targeted control strategies aiming to improve udder health in dairy cattle. We hypothesised that integrating contact networks with high-resolution sensor data and strain-level pathogen information enables the identification of transmission routes and the individual contribution of each cow to pathogen transmission. The objective of this study was to apply a previously developed likelihood-based computational framework to contact network data from a commercial dairy operation to elucidate transmission dynamics of Staphylococcus aureus (S. aureus) in the herd. Data were obtained from 250 lactating cows monitored over five months in a commercial dairy barn equipped with a real-time location system (RTLS) that recorded positions at 1-s intervals. The RTLS provided information on proximity contacts, area utilisation similarity, and sequential cubicle occupancy, which were integrated with milking parlour records specifying the time and stall position of each cow at each milking event. Concurrently, quarter-level milk samples were collected at bi-weekly intervals, and cultured S. aureus strains were typed using a randomly amplified polymorphic DNA (RAPD)-PCR test. The sensor information was used to create dynamic transmission networks that could explain the transmission of S. aureus from infected to susceptible cows between the sampling time points. These networks were constructed from proximity data, area utilisation similarity, and sequential use of milking units and resting cubicles. Transmission dynamics were modelled using an individual-based stochastic SIS (Susceptible–Infected–Susceptible) framework on candidate contact networks, where each cow's infection risk was determined by network-derived effective contact exposure to infected cows and by S. aureus–specific transmission and recovery parameters (β, γ) estimated from longitudinal bacteriological milk-sampling data. The likelihood of the observed infection sequence was computed, and networks were compared using maximum likelihood estimation to identify the most plausible transmission pathway. Individual-level metrics were derived to quantify transmission potential (risk-transmission index, defined as the proportion of effective contacts relative to total contacts), susceptibility, and resilience, accounting for the frequency, duration, strength, and spatial context of effective contacts with infected individuals. The inferred transmission patterns revealed substantial differences in likelihood across transmission networks and in their relative contributions to the endogenous persistence of S. aureus within the herd. The role of infected cows was evaluated individually across proximity-based, milking-order, and cubicle-use transmission pathways, revealing marked heterogeneity in their contribution to effective transmission events. These findings demonstrate that combining sensor-derived contact information with a stochastic network-based modelling framework enables reconstruction of biologically plausible transmission routes. The proposed framework provides a quantitative basis for identifying high-risk individuals and transmission pathways within barns, thereby supporting the development of targeted mastitis control strategies based on precision livestock data.

Responses of dairy cow to common stressors: effects on milk production and feeding behaviour traits

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The efficiency of the dairy livestock sector is a fundamental point given the near future worldly challenges, and precision farming surely is a useful tool to guarantee an adequate efficiency level. In this field, the high-frequency automatic data collection and interpretation represent the key point to address an optimal production level. Several aspects could be considered, but the feeding behaviour is one of the most monitored traits because it is closely linked to two main aspects as feed efficiency and animal welfare. Nowadays, this aspect is easy to monitor given the widespread adoption of technology at several production stages, integrated in various devices present in commercial farms. The presence of a stress condition in dairy cows generally leads to a modification in normal feeding behaviour, reducing the eating time (ET) and the rumination time (RT). The most common primary sources of stress in dairy cows are mastitis (M) and lameness (L), but heat stress (HS) surely also plays an important role among stressors. Solutions to assess drops in milk yield under stress conditions (MY) have been extensively studied, but models to quantify changes in animal behaviour, mainly ET and RT, are still lacking. The current study tries to contribute in this sense, adopting a smoothing approach to identify and characterise perturbations in three different aspects ET, RT and MY. Raw data were collected from 350 Italian Simmental cows in some dairy farms in north-east Italy provided with an automatic milking system and accelerometers, and within this population, cows with a lactation period of at least 150 days were selected. To evaluate the response time, the recovery time, and to quantify the production and feeding behaviour losses, a double-curve approach was used. Differences between the two curves characterised by different smoothing parameters ($\lambda = 100$ and $\lambda = 10{,}000$) were fitted with a linear mixed-effect model. The lactation stage (early, mid, late), parity (primiparous, multiparous) and perturbation cause (M, L, HS) were considered as fixed factors while the effect of farm and cow (nested within farm) were included as random. The p-values for multiple comparisons were adjusted using the false discovery rate method. Results obtained indicate that L generates the longest (30.6 and 28.8 d, respectively) and most difficult perturbations to recover, both for MY and ET. Moreover, it also caused the greatest loss in milk production (14.7 kg). The HS caused the greatest losses regarding feeding behaviour (175.2 and 210.3 min, ET and RT, respectively). Overall, seems that M had a lower impact on aspects considered. Primiparous cows showed faster responses to stress but slower recovery times compared to multiparous ones. However, multiparous exhibited a greater total MY losses. The method applied has been shown to be effective to quantify losses and recovery time at individual level proving to be an effective solution to evaluate response of each animal to physiological and environmental events adding useful information to enhance the livestock sustainability.

Innovative functional modelling framework reveals black globe temperature as the most robust predictor of circadian heat related behavior in grazing dairy cows

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Capturing 24-h behavioral responses to heat stress remains analytically challenging due to strong non-linearities, individual variability, and the temporal structure of grazing systems. Traditional analyses typically ignore circadian dynamics or assume linear thermal responses, limiting their capacity to detect biologically relevant shifts. We aimed to develop an advanced modelling framework capable of (i) identifying which climatic index best predicts circadian behavioral rhythms under pasture conditions, and (ii) quantifying how rhythms re-time under hot vs. cool days. We hypothesized that black globe temperature (TBG), integrating radiation and air temperature, would outperform other indices. We analyzed 2.46 million accelerometer-based behavioral records collected at 5-min intervals from 86 grazing Holstein cows over two summers. Eight climatic indices were derived from hourly meteorological data. Circadian dynamics of twelve behavioral traits were modelled using big additive models (BAM) with cyclic cubic splines, allowing smooth 24-h periodicity and scalable computation on large datasets. Each climatic index was tested via a dedicated spline term, and performance was compared using AIC. Days were classified into cool (≤23.6 °C TBG) and hot (≥29.4 °C) based on empirical quantiles, and BAMs were refitted for each class to quantify functional differences in rhythm amplitude, phase, and shape. Confidence bands were used for inference on whole-curve differences rather than pointwise values, enabling functional comparison of circadian patterns. TBG provided the lowest AIC in 10/12 behaviors, clearly outperforming THI, CCI, HLI, WBGT and other indices. Functional predictions revealed significant, systematic re-timing of behaviors between hot and cool conditions, as evidenced by non-overlapping 95% simultaneous confidence intervals across extended time windows. Under heat, cows shifted rumination and resting towards the night–early morning, increased standing in early morning and late afternoon, and reduced midday activity—patterns consistent with circadian thermoregulatory adjustments. These results demonstrate that the BAM-based functional approach uncovers subtle rhythmic reorganizations that would remain undetected with classical linear or mean-based analyses. This study introduces a novel, scalable functional modelling pipeline combining BAM, cyclic splines and quantile-based thermal classification to dissect circadian behavioral responses at high temporal resolution. Black globe temperature emerged as the most reliable environmental driver of circadian dynamics in grazing cows, highlighting the central role of solar radiation in shaping heat-related behavior. This framework offers a methodological advance for heat-stress research and provides a transferable tool for evaluating thermal stress in precision livestock systems, especially where large behavioral time-series and complex non-linear rhythms must be analyzed.

Impact of Weaning and THI on Dairy Calf Behavior

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Weaning is a critical phase in the management of dairy calves, characterized by significant changes in behavior and feed intake, which can be strongly influenced by ambient temperature. However, the extent of this impact remains unclear. The use of electronic monitoring devices can provide detailed assessments of animal behavior, feed intake, and the temperature-humidity index (THI). This study aimed to evaluate behavioral (activity and inactivity) and feeding (concentrate and water intake) changes in dairy calves during weaning and to investigate the influence of the temperature-humidity index (THI) on these variables. Thirty-seven male Holstein calves were monitored during weeks 7 (pre-weaning), 8 (start of weaning), and 9 (weaning progress). Calves were fed milk replacer via an automated liquid diet feeder, and their housing was equipped with automated concentrate feeders and water drinkers to allow individual monitoring. Milk replacer was offered at 6 L/day (840 g total solids) until 49 days of age (week 7), then reduced to 4 L/day (560 g total solids; week 8) and 2 L/day (280 g total solids; week 9). Activity and idleness were continuously recorded using commercially available collars fitted to calves throughout the weaning period. Behavioural data were collected hourly and then grouped into time periods (morning, afternoon, and evening), while intake data were recorded daily. THI was recorded throughout the experimental period (average 73.3, maximum 80.0 and minimum 61.0). Multivariable mixed models were used to analyze associations between week of life (7, 8, and 9) and behavioral and feeding variables, with week 7 as the reference because it preceded the onset of weaning. Activity increased by 12.55 min/day in week 8 ($P = 0.0083$) and 42.34 min/day in week 9 ($P < 0.0001$) compared to week 7. Idleness decreased similarly, by 12.54 min/day in week 8 ($P = 0.0083$) and 42.33 min/day in week 9 ($P < 0.0001$). Activity was higher and idleness lower in the morning and evening than in the afternoon, with differences of 11.99 and 17.70 min/day, respectively. In addition, for each one-unit increase in THI, activity increased by 4.34 min/day, with a corresponding decrease in idleness ($P < 0.0001$). Concentrate intake increased by 0.41 kg/day in week 8 and 1.09 kg/day in week 9 ($P < 0.0001$) compared to week 7. Each one-unit increase in THI was associated with a 5.6 g/day decrease in concentrate intake ($P = 0.0302$). Water intake increased by 1.24 L/day in week 8 and 3.27 L/day in week 9 ($P < 0.0001$), along with an additional 74.5 mL/day increase for each unit rise in THI ($P < 0.0001$). The observed increase in activity and reduction in inactivity following the onset of weaning suggest heightened restlessness in calves, likely associated with foraging behavior and dietary adaptation due to reduced milk supply. Weaning induced significant behavioral and feeding changes, which were further modulated by THI, highlighting the importance of environmental management for calf welfare. FAPESP 2020/15745-5 and 2021/07131-0, CNPQ 301990/2022-0

Quantification of resilience indicators in broiler chickens through the analysis of growth and feed intake dynamics

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Improving the resilience of broiler chickens is increasingly important to ensure sustainable poultry production under external challenges such as climatic variability or pathogen exposure. Resilience is a multifaceted biological trait defined as the ability of an animal to withstand, adapt to, and recover from environmental or physiological perturbations while maintaining productive performance. Because resilience cannot be measured directly using conventional end-point traits, innovative quantitative approaches based on longitudinal data are required. The objective of this study was to develop and validate a methodology to quantify key traits of resilience in fast-growing broiler chickens by analysing the dynamics of body weight (BW) and cumulative feed intake (CFI) in response to a perturbation. The approach relies on modelling performance trajectories and characterising stressor-induced deviations. First, non-perturbed ("reference") trajectories for BW and CFI are estimated by fitting appropriate nonlinear models to longitudinal time-series data. An iterative procedure based on residual autocorrelation analysis progressively excludes perturbed observations until no significant autocorrelation remains. Deviations between observed data and the corresponding reference trajectory are then quantified, and their temporal dynamics are described using an asymmetric Gaussian function. The response to a perturbation is characterised using six complementary parameters: (i) maximum deviation amplitude, (ii) time of maximum deviation, (iii) onset time, (iv) recovery time, (v) skewness of the deviation curve, and (vi) the integral under the curve representing cumulative impact. Together, these parameters provide an integrated description of both resistance to perturbation and recovery capacity, two major components of resilience. The methodology was evaluated using data from a controlled coccidia infection challenge (Eimeria maxima) in broiler chickens fed diets diluted with increasing levels of lignocellulose. Although CFI did not differ significantly between diets overall, resilience analysis revealed marked differences in response dynamics. Compared with the control diet, birds fed the most diluted diet (HF; 15% lignocellulose) showed a significantly lower maximum deviation for both CFI and BW (−4% vs. −11%, $P < 0.001$), as well as a lower cumulative impact, assessed by the integral of the deviation curve (CFI: −17 vs. −50 %·d; BW: −21 vs. −63 %·d). In addition, HF birds exhibited a shorter perturbation duration for BW (8.7 vs. 11.1 d; $P < 0.05$) and a delayed onset of the BW response (4.7 vs. 3.8 d; $P < 0.01$), further supporting a more resilient response. Overall, analysing BW and CFI dynamics provides a robust framework for quantifying resilience in broiler chickens. The proposed methodology can be applied at both individual and flock levels and offers promising applications for genetic selection of more resilient animals, as well as for evaluating nutritional or management strategies aimed at improving robustness and adaptability in poultry production systems. In this perspective, the method will soon be applied to individual longitudinal data from fast-growing chickens exposed to a thermal challenge (heat stress).

Using ultra-wideband tracking technology to monitor activity and area use in broiler breeders

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In Europe, broiler breeders are commonly housed in large flocks in floor/slat systems. Within these flocks, the interaction between male and female birds is of great importance for the production of fertilized eggs. However, manually monitoring the behaviour of individual broiler breeders is very challenging, due to their large numbers and similar appearance. Here, an ultra-wideband (UWB) tracking system was implemented to record the location and movement of individual broiler breeders kept in a commercial house (59 x 14 m) in a flock of 5,950 birds (350 males and 5,600 females). The house was physically separated in two identical – but mirrored – halves. At 23 weeks of age, 56 broiler breeders (n = 28 male, n = 28 female; in a 50/50 split across the two sides of the house) were fitted with an UWB tag in a backpack. The body weight of all birds was recorded, and the comb (on a scale from 1 to 3) and overall condition (scale 1-5) of males were scored. For females, the feathering (scale 0-5), pinbones (scale 0-3) and wing feathers (scale 0-10) were scored. The UWB tags that were fitted to the birds sent out a signal to receivers in the environment, with a preset sampling rate of approximately one sample every second, although the realized average sampling frequency was lower. The location of the birds was recorded continuously throughout the house, and we here studied the first subset of data from one month of tracking (23-27 weeks of age). During this time span five birds lost their tags, resulting in a remaining sample size of 51 birds (n = 24 females, n = 27 males). Three indicators of activity and area use were determined: 1) distances moved (i.e., the Euclidian distance between subsequent detections of the same bird), 2) functional area use within the house (i.e., the proportion of detections of a bird on the litter, on the slats and in the nest box area), and 3) overall area use (i.e., the proportion of the total house area visited in a day, for which both halves of the house were virtually split into 20 zones). In the analyses, a potential effect of side of the house was accounted for and preliminary results are presented here. An overall average daily distance moved of 2,141 (SD 654) m was observed. Daily distances moved were higher for males than for females (model estimate of 2,435 versus 1,795 m, $p < 0.001$). Within males, no effects of body weight, comb or overall condition on distances moved were observed, and within females, no effects of body weight, pinbones or feathering (no variation present; all females had the same feathering score) were observed (all $p > 0.05$). A link with wing feather score was observed for distances moved ($p < 0.001$), but given the small sample size per feather score, this requires further investigation. In terms of functional area use, males spent more time in the litter area than females did ($p < 0.001$), while females spent more time on the slats and in the nest box area than males ($p < 0.001$and $p = 0.009$, respectively). The overall average number of zones visited was observed to be 14.9 (SD 3.8) out of 20 (approx. 75% of the available area). No difference was observed between the sexes ($p > 0.05$), but the number of zones visited decreased during the month of recording (estimate = -0.04, $p < 0.001$). Overall, these preliminary results indicate that males and females use the house differently, which can have consequences for the interaction – and reproduction – of males and females. Next steps include further investigation of individual differences and interactions between males and females.

Correspondence-Based Explainable Deep Learning for Automated Recognition of Pre-Competition Stress in Horses

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Stress in horses is a multifactorial physiological and psychological response to environmental and management challenges and is particularly relevant in competitive sport settings. Conventional assessment relies on physiological measurements and manual behavioural coding frameworks such as the Equine Facial Action Coding System (EquiFACS), which provides anatomically grounded facial motion descriptions but requires trained coders and is difficult to implement for continuous monitoring. This proof-of-concept study explored whether deep learning models can distinguish relaxed and pre-competition tense states from video recordings and whether a correspondence-based explainability framework (SHIC-XE) can highlight anatomically meaningful regions consistent with EquiFACS-based descriptions and expert-defined regions of interest. Video data collected under baseline and pre-competition conditions were processed using automated detection, segmentation, and transfer-learning-based feature extraction. A supervised neural network classifier was evaluated using leave-one-horse-out cross-validation to assess generalization across individuals. Model performance was quantified using accuracy, precision, recall, F1-score, sensitivity, and specificity at frame and video levels. To improve interpretability beyond conventional 2D saliency maps, gradient-based explanations were projected onto a canonical 3D equine head model via dense 2D–3D correspondence mapping, enabling viewpoint-invariant and temporally coherent aggregation of attention. Regional attention distributions were compared across behavioral states and examined in relation to EquiFACS-informed anatomical regions and expert visual assessment. Preliminary findings suggest that automated classification can differentiate relaxed and tense states under competition-related conditions. Whole-body representations tended to provide stronger discrimination than facial crops alone. Correspondence-based explainability indicated increased attention to supraorbital and auricular regions during tense states, broadly consistent with documented stress-related facial movements. These results provide initial evidence that correspondence-based explainable AI may offer a complementary tool to manual coding frameworks for equine stress assessment and could contribute to future development of transparent decision-support systems in sport horse welfare monitoring.

Fear reactions in cattle, sheep, and horses to potential predator-deterring stimuli: Does a predator stimulus induce fear-enhanced startle in livestock?

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As large predators increasingly re-emerge in Western and Central Europe human-wildlife conflict becomes more pronounced and effective predator deterrence gains importance. Therefore, the aim of the mAInZaun project was to develop an 'intelligent' fence, that – using artificial intelligence – is able to automatically detect and deter wolves when they approach livestock pastures. However, when predators are deterred from pastures housing livestock, such deterrence stimuli must not be harmful to the livestock themselves. Therefore, the aims of the project were (1) to test the effects of potential predator-deterring stimuli on startle and fear reactions in cattle, sheep and horses and to compare these reactions to reactions to a known startle stimulus and (2) to assess potential fear-enhancing effects of predator stimuli on startle reactions in cattle, sheep and horses. The experiment included three studies, involving 20 cattle, 60 sheep and 20 horses, respectively. Animals were exposed in balanced order to the following potential predator-deterring test stimuli, each presented with and without a predator stimulus (recording of wolf howling): umbrella (serving as a known startle stimulus), strobe light, ultrasound, a scent marketed as wolf-deterrent (cattle only) and spraying from a spray bottle (horses only). Animals were brought to a test compartment either alone (cattle, horses) or in groups of five (sheep). They were allowed a habituation period, were then allowed to feed from a trough with concentrates and were then exposed to the startling stimuli while feeding. The intensity of startle and fear reactions was measured via a behaviour score, latency to resume feeding, heart rate (sheep and horses), heart rate variability (horses) and thermal images of the eye (cattle). Additionally, behaviour patterns such as vocalisations, elimination and freezing were recorded. As expected, mixed model analyses revealed numerically stronger reactions to the umbrella in all three species, compared with the other stimuli, although not all pairwise comparisons were significant (e.g. cattle, umbrella-strobe light: $P>0.1$ for the behaviour score), indicating that some of the potentially wolf-deterring stimuli should only be used after habituation of livestock to these stimuli. For the majority of parameters and stimuli, presentation of the wolf stimulus alongside the test stimuli did not result in greater startle reactions. However, sheep showed significantly greater startle reactions to the strobe light and the umbrella, when these were presented with rather than without predator stimulus (score: $P<0.05$). Cattle tended to react mores strongly to the scent and the ultrasound and also tended to have higher eye temperatures when presented with rather than without the predator stimulus (all $0.05<P<0.1$). For horses, no such clear trend regarding the predator stimulus was observed. Results indicate that, while livestock reacted to the predator-deterrence stimuli with startle and fear reactions, reactions were generally not pronounced, and habituation should be possible. However, the presence of wolf stimuli may induce fear-enhanced startle reactions, particularly in sheep. Therefore, potentially wolf-deterrent stimuli intended for use in livestock pastures should not only be tested in isolation for potentially harmful effects on livestock, but also in combination with predator stimuli to better mimic real-life situations.

How can we effectively improve the welfare of broiler chickens in commercial production systems? A review of farming practices and their relationship with broiler welfare

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With growing attention to sustainable farming practices, broiler production systems are becoming increasingly diverse and often adopt a range of welfare promoting management strategies. Several countries have already undergone a national transition toward systems that promote higher broiler welfare, each country implementing its own set of requirements. A broader shift at the European level is also expected, for example through the uptake of the European Chicken Commitment by food businesses. Higher-welfare broiler production systems differ substantially in their specifications, ranging from indoor systems with relatively small reductions in stocking density and some environmental enrichment to highly extensive systems that include outdoor access and the use of slow-growing strains. For some of the management measures commonly applied in commercial higher-welfare systems, a substantial body of research is available to support the choices made, particularly regarding reduced stocking density and the use of slower-growing broiler strains. However, for other measures, such as early post-hatch feeding, specific lighting programs, the combination of different enrichment types or a covered veranda, as well as practices related to catching and transport, scientific evidence is more limited. Moreover, interactions between these factors and key variables such as stocking density or breed remain poorly understood, e.g., whether fast- and slow-growing strains have different requirements or responses to the environment. This presentation will evaluate key management approaches frequently used in higher-welfare broiler systems in terms of their effects on broiler welfare and will identify areas where further research is needed. Promising management factors that could be incorporated into future higher-welfare systems will also be highlighted. Because management factors may interact and commercial conditions often differ markedly from those in experimental or small-scale studies, research under controlled conditions must be validated by data collected in commercial practice. Moreover, comparative studies of production systems based on welfare outcomes provide valuable insights that cannot be obtained from experiments focusing on single factors. We will illustrate this with examples of system comparisons with respect to broiler welfare outcomes. Finally, we will consider whether current higher-welfare broiler systems not only reduce negative affective states but may also promote positive affective states by offering birds greater choice and rewarding experiences. Potential avenues for future research in this area will be discussed.

Early-life characterisation of temperaments in pullets in relation to subsequent severe feather pecking behaviour

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Injurious pecking in laying hens has a multifactorial and episodic origin, making its causes difficult to identify and control. Severe feather pecking can cause cannibalism, high mortality, and reduced egg production, with important animal welfare and economic consequences. Beak trimming — the cauterisation of the beak tip using an infrared laser — has long been used to limit the associated damage, but is considered potentially painful and has been banned in several European countries. Notably, bans have been implemented primarily in countries where flocks consist mainly of white egg-laying strains, which are generally less prone to pecking than the brown strains predominant in France. Alternative strategies such as environmental enrichment, light or nutritional management have been studied, but show variable effects and generally only mitigate the consequences of injurious pecking once it emerges. Beyond management factors, individual behavioural traits may also influence susceptibility to injurious pecking. Hens vary in exploration, learning, and stress responses along a proactive–reactive axis: proactive individuals tend to be bold and active, whereas reactive individuals are more cautious and flexible. Proactive traits have been associated with higher feather-pecking propensity and appear partly heritable, suggesting that temperament may play a role in pecking behaviour. Yet the full behavioural profile of hens in relation to their pecking propensity remains poorly understood. We thus aimed to determine whether behavioural – proactive or reactive – traits in pullets predict later occurrence of injurious pecking. A total of 200 ISA Brown hens were monitored under commercial-like conditions. A battery of behavioural tests was conducted at two pullet stages (5–7 and 12–15 weeks) and at the onset of lay (17–21 weeks) to assess temperamental traits, based on tonic immobility, attention bias, social motivation, emergence, and food perseverance tests, alongside individual in situ activity (29 observation days x 3 scans per day, totalling 69 scans) and body weight. Severe feather pecking during the laying period was recorded for each hen during two daily 15-minute sessions over 33 days (weeks 30-36), and used to classify individuals as high, moderate or low peckers. The relationship between this classification and early-life behavioural profiles is investigated to identify profiles associated with a higher propensity for pecking. Data analyses are ongoing and will be finalised for presentation. Early behavioural predictors could support development of screening tools for farmers to implement preventive measures from the pullet stage onwards.

Effects of drinking water optimization on immune, physiological welfare parameters, egg quality, and productivity in laying hens

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Maintaining physiological stability (homeostasis) is essential for animals' survival. Accordingly, any event that disrupts homeostasis is defined as stress and poses a threat to survival, and coping with this threat and restoring physiological stability requires substantial physiological resources. Restoration of homeostasis is made possible by the synergistic activity of many physiological systems, among which the immune system is one of the main and most important. Animals raised under intensive farming conditions are exposed to many stressors. For several years, our laboratory has been developing a minimally invasive method for measuring stress, in order to provide reliable and quantitative markers of stress experienced by animals during rearing. The need for such a method arose for two main reasons: first, concern for animal welfare, as the method makes it possible to identify stressful management conditions with the aim of improving them; and second, the need to detect stressful management conditions that may impair animal performance during rearing. To date, the method has been applied to measuring stress in chickens (layers and broilers) and turkeys under various management conditions, such as stocking density, molting, feed withdrawal, transportation, and marketing. Here, using the same methodology, we sought to assess the stress status of commercial Lohmann laying hens under two watering systems (treated water versus tap water). Two hundred layers were housed in eight community cages. Each cage contained 25 hens at a stocking density of 750 cm² per hen. Half of the cages received treated water (acidified and chlorinated) and the other half received untreated tap water; water and feed were available ad libitum. The experiment consisted of three biological replicates. Each replicate began with a 3-week recovery period, followed by allocation to groups, blood sampling after 3 weeks of treatment, and then repetition of this sequence. In each biological replicate, blood was sampled from 15 different hens from each treatment group. We found that while there were no changes in physiological parameters such as hematocrit and total protein, there was a significant ($p<0.05$) decrease in oxidative-active metabolites (AGEs) and in the antioxidant protein lysozyme serum levels, as well as a decrease in pro-inflammatory cytokines IL-6 and IL-1β and stress-related markers Heat Shock Protein 70 (HSP-70) and glucocorticoid receptor (GCR) gene expression in peripheral blood leukocytes (PBLs) in the treated-water group. Additionally, a reduction in pathogenic bacterial burden in the drinking water, alongside improvement in most productivity and egg quality parameters, was recorded in the treated-water group. In light of these observations, it appears that laying hens undergo a process of coping with oxidative stress through elevated levels of immune antioxidant metabolites. Moreover, optimizing hens' drinking-water quality seems to have stress-reducing and beneficial effects.

Enhancing laying hen welfare and thermal comfort in cage-free systems using cooled perches.

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Housing conditions are critical determinants of laying hen welfare. While conventional cages restrict natural behaviors and impair skeletal health, cage free systems allow expression of natural behaviors but introduce problems such as feather pecking and keel bone injuries. Environmental enrichment in cage-free systems, particularly perches, is essential for enabling natural resting behavior, reducing aggression and supporting bone strength. This study investigates an innovative approach to improve the thermal comfort, health, and welfare of laying hens within cage-free systems. We evaluated the efficacy of cooled perches in aiding thermal regulation in laying hens during periods of intense heat stress. The study was conducted over the summer months (July-September) in an experimental cage-free system, and involved 50 laying hens housed at a density of 7 hens/m². The pen was equipped with feeders, drinkers, laying nests and two sets of perches elevated 70 cm above the littered floor. One set was actively cooled to 24°C using circulating water. Following an initial adaptation period, the pen was divided into two sections, with each section containing one type of perch. To gather physiological data, internal data loggers were implanted in 24 hens (12 per group), continuously recording core body temperature in 20 hens, and both temperature and heart rate in 4 hens. Behavioral observations were captured via continuous video recording, supplemented by intermittent thermal imaging to measure feet temperatures on both cooled and control perches. Throughout the experiment, high environmental temperatures and humidity were recorded, creating significant heat stress, making the study highly relevant to real-world challenges. The cooled perch system consistently maintained its target temperature of 24°C. Our physiological data revealed that laying hens with access to cooled perches consistently exhibited lower core body temperatures (0.3-0.5°C cooler) and more stable thermal profiles compared to hens in the control group. This indicates efficient heat conduction from their feet to the cooled perches. Behavioral analysis from video recordings further supported these findings: hens in the cooled perch group spent significantly more time on the perches, particularly during hot afternoons and evenings, demonstrating a clear preference and enhanced comfort. In contrast, control group hens tended to cluster on the litter. The even distribution of hens on the cooled perches suggested genuine thermal relief, unlike the control group where hens gathered near drinkers, likely seeking water to cope with the heat. Thermographic imaging provided visual evidence, showing lower contact temperatures (≈27–29°C) on cooled perches compared to control perches (≈30–31°C), coupled with reduced foot temperatures, underscoring the effectiveness of heat transfer. Laying rates were similar in both groups and were not affected by the cooled perches. The findings of this study demonstrate that cooled perches significantly enhance both the thermal welfare and behavioral comfort of laying hens in hot climates. By providing an effective mechanism for heat dissipation, cooled perches offer a viable and sustainable solution for managing heat stress, promoting better health outcomes, and ultimately improving the overall welfare of laying hens in cage-free poultry farming systems and specifically in houses without thermal regulation.

Welfare indicators during the early rearing period – Comparing young birds of dual-purpose and layer hybrid genotypes under organic conditions

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Dual-purpose chicken genotypes (DPG) are considered a more ethical alternative to intensive layer and broiler systems, being more robust with good welfare performance. However, most welfare evaluations are conducted at the end of the fattening or during the laying period and little attention is paid to the rearing period. This phase is a critical developmental window, where early-life conditions have lasting impacts on health and productivity. Understanding welfare development during this period is essential for designing management strategies that support high standards throughout the production cycle. In this context, we investigated two DPG (Coffee, Cream) and one layer hybrid (ISA Warren) from hatch to 6 weeks of age (woa) under organic conditions (approval no. IX552-105434), assessing genotype-, sex-, and age-related differences in welfare indicators. Chicks of Coffee (COF n=333) and ISA Warren (WA1 n=352) hatched in October 2024, and Cream (CRE n=353) and ISA Warren (WA2 n=345) in November 2025; each was individually wing-tagged at placement. Two weeks later, each genotype was split into even numbered, mixed-sex groups. Birds were reared on deep-litter floors with perches, provided maize silage, dustbaths, and a lactic acid supplement, with access to a covered veranda from 4 woa. Organic feed was supplied according to standard nutrient requirements, alongside standard lighting, temperature, and vaccination protocols. Welfare indicators were assessed at 2/3 and 6 woa using the MTool protocol, covering 14 body regions on an ordinal scale (0–2), and birds were weighed at each assessment. BW and welfare scores were analysed using a general linear model with Tukey and Bonferroni adjustments respectively, including genotype, age, sex, and their interactions as fixed effects, with wing band as random effect. DPG exhibited higher growth performance than the layer hybrid. At the end of the mixed-sex rearing period at 6 woa, BW was highest in COF (791g), followed by CRE (599g), WA1 (518g), and WA2 (467g). Sex significantly affected BW, with the largest male–female differences observed in the DPG (CRE 22%, COF 20%) and the smallest in WA2 (17%), indicating stronger sexual dimorphism in DPG already evident in early BW development. Despite these growth differences, genotype effects on welfare were not uniform across indicators. Only three out of 14 welfare indicators – pecked and used wing feathers as well as footpad health – consistently differed between genotypes, with WA1 and COF showing the best outcomes. For pecked and used wing feathers, a significant sex*week interaction was observed, whereas sex alone had no effect. Pecked feathers on the tail or back showed a significant genotype*age interaction, with CRE having increased scores at 3 woa that resolved by 6 woa. Footpad health was influenced by both genotype and age, with WA2 showing the poorest outcome. Age, and its interaction with sex or genotype, significantly affected several welfare indicators, emphasizing the importance of age-specific management during rearing. In contrast, genotype effects were indicator-specific and generally less consistent across the tested genotypes. Behaviour-related influences on indicators can emerge during the rearing period, locomotor activities such as perching and exploration should be encouraged and guided to support healthy development. The results demonstrate that welfare in young chickens is dynamic and shaped by interacting effects of age, genotype, and sex. Understanding how welfare parameters change over time can improve management strategies that consider both genetic background and rearing conditions, laying the foundation for healthy, robust, and resilient chickens.

Precision farming practices for sustainable poultry production in the USA

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The global population is predicted to reach over 9.5 billion in 2050, by then the requirement for animal protein (e.g., eggs, meat, and milk) will increase by over 70% as compared to 2005. Providing food for the increasing world population with limited natural resources is a grand challenge for animal agriculture. The animal farming systems in U.S. play a key role in feeding the increasing world population. According to USDA, the total US poultry sector sales in 2025 were close to $90 billion (e.g., 9.3 billion broiler chickens and 100 billion table eggs), which ranks the US one of world's largest poultry and egg production countries. Despite this productivity, concerns remain regarding animal welfare and environmental conditions in poultry production systems. Fast-growing broiler chickens frequently experience welfare issues, with about 30% suffering from leg problems and over 50% developing footpad lesions. In egg production systems, cage-free laying hens have been reported to experience nearly double the mortality and injury rates compared to hens raised in conventional cage systems. Additionally, air quality challenges in cage-free housing systems continue to raise concerns for both animal welfare and worker health. To improve sustainability and address these issues, precision animal farming technologies are increasingly needed. In this study, artificial intelligence (AI)–based technologies, including machine vision and robotics, were developed to monitor and manage poultry health and welfare. These advanced systems achieved over 90% accuracy in detecting conditions such as footpad dermatitis, bumblefoot, and bird mortality. Furthermore, large foundation models such as SAM (Segment Anything Model) and TAM (Track Anything Model) were effectively applied for individual bird tracking and locomotion speed monitoring. For improving air quality management, electrostatic airspace charging systems and electrolyzed water sprinklers were designed and evaluated in commercial cage-free houses, demonstrating promising control efficiency.

A novel method to evaluate the social impact of livestock systems: a social life cycle assessment of the impacts of the Irish pig value chain

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The social sustainability pillar of livestock systems is the most commonly overlooked amongst the three pillar of sustainability. Social Life Cycle Assessment (S-LCA) is widely used to evaluate social sustainability, yet its application to the assessment of social impacts in agri-food systems has notable limitations. These include reliance on social performance assessments, lack of sector-specific activity variables and use of linear reference scoring, which can distort the representation of social impacts in such systems. We developed an enhanced S-LCA framework that integrates social risks and hotspots with social performance, incorporates added-value activity variables tailored to agricultural systems and replaces linear scoring with contextualized nonlinear scores. The Irish pig value chain is used as a case study. We added pigs as a Stakeholder category, alongside existing Stakeholders of S-LCA. Performance reference points (PRP)and inventory indicators were derived from national and regional pig industry benchmarks. Social performance was evaluated by comparing each indicator with its corresponding PRP and assigning an ordinal score. These ordinal scores were converted into cardinal through expert judgement to enable nonlinear scoring. Both ordinal (linear) and cardinal (nonlinear) scores were transformed into social risk values (S_i) using reversed min–max normalization, such that $0 < S_i < 1$, where a value of 1 represented extreme social risk. Adapted time-based added-value activity variables (A_i) were defined for stakeholder groups as follows: “people days” for the local community and society, “consumption days” for consumers, and “pig life days” for pigs. Impact sub-categories were ranked by experts, and their relative weights (W_i) were derived using the Analytic Hierarchy Process. Social risk time (SRT_i) for each inventory indicator was calculated as $S_i \times A_i \times W_i$ and expressed as a percentage relative to the aggregated value for each stakeholder group. Social hotspots were estimated using SRT_i / SRT□, where SRT□ corresponded to the case where S = 1. Social risks (S) were highest for pigs (0.57 vs. 0.64) and lowest for society (0.47 vs. 0.44) under the linear and nonlinear methods, respectively. The SRT differed between scoring approaches across all stakeholder groups. Linear scoring overestimated overall SRT for society (3.5%) and consumers (67.0%) and underestimated SRT for the local community (4.7%) and pigs (11.1%). Both methods identified the pig farm (0.57 vs. 0.65) and local community (0.52 vs. 0.55) as social risk hotspots and the consumption stage (0.39 vs. 0.31) as a social opportunity hotspot. The linear scoring method showed systematic bias in estimating social risk, whereas incorporating contextualization in the non-linear scoring produced more nuanced and granular results across all stakeholder groups. This implies that linear scoring tends to misrepresent social issues in pig value chains because it applies a uniform scale to all performance levels. The proposed methodology highlights the importance of assessing social risks and hotspots alongside social performance in agricultural S-LCA. Incorporating time-based value-added activity variables specific to agricultural system and developed for each stakeholder group improves the representation of exposure duration to social issues and more accurately reflects how value-chain processes shape social impacts. The study also demonstrated the advantages of nonlinear scoring over the linear method.

Impact of animal welfare on milk production and economic performance in dairy farm

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Animal welfare is increasingly recognized as a key component of sustainable livestock farming, yet its quantitative contribution to production efficiency and farm profitability remains insufficiently documented. This study assessed the relationship between animal welfare, milk production, and economic performance in dairy farms using a farm-level quantitative approach. A total of 169 dairy farms were evaluated using the ClassyFarm protocol, which integrates animal-based, resource-based, and management indicators into a composite welfare score. Farms were classified into low (<50), medium (51–60), and high (61–81) welfare categories. Milk yield, somatic cell score, milk quality traits, and energy-corrected milk (ECM) were analyzed in relation to welfare level, housing system, breed, herd size, and access to grazing. Multivariable regression models were applied, and causal effects of higher welfare standards were estimated using regression adjustment and augmented inverse probability weighting (AIPW). Farms with higher welfare scores showed significantly higher milk yield and ECM, alongside lower somatic cell scores, compared with low-welfare farms. Free-stall housing and access to grazing were positively associated with ECM, while breed-specific responses highlighted differences in performance under varying welfare conditions. Causal estimates indicated that adoption of higher welfare standards increased ECM by approximately 1.8–2.0 kg per cow per day, corresponding to an additional economic return of 3.2–3.6 € per cow per day. These results demonstrate that improved animal welfare contributes positively to both production efficiency and economic sustainability in dairy farming systems. Integrating welfare-oriented management strategies can therefore enhance farm resilience and profitability while supporting a more sustainable livestock sector.

From live animal export to meat and carcass trade: economic, sustainability, and welfare perspective

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Long-distance live animal transport continues to raise significant animal welfare, environmental and public health concerns. Drawing on a comparative cost-benefit trade modelling framework, this study demonstrates that transitioning to meat and carcass trade is economically feasible, reduces the environmental footprint associated with long-distance transport, and strengthens the competitiveness and resilience of the EU agri-food sector. The study synthesises evidence from two completed analyses—a Portugal-Israel trade case (sheep) and a Romanian live export assessment (bovine and ovine)—and two ongoing projects—unweaned calf transport from Ireland to Spain, and lamb exports from Romania to Italy. For each case study, two scenarios are evaluated: long-distance live animal export and meat and carcass trade. Key indicators include: transport duration and costs, mortality and morbidity, abattoir throughput, value-added retention, fuel consumption and CO_2-equivalent emissions, including cold-chain logistics. Scenario outcomes are compared across economic, welfare, and environmental dimensions to assess the feasibility and impact of transitioning to carcass-based trade. The economic modelling indicates that carcass trade can offer structural and economic advantages. In the Portugal-Israel case, exporting carcasses was estimated to cost nearly 2.5 times less per kg than live animals, while enabling higher utilisation of domestic slaughter capacity and retention of value-added processing within the EU. Romanian modelling suggests that phasing out live exports could generate approximately 5,300 jobs and €46 million in gross value added. Environmental assessment indicates lower impacts under carcass trade scenarios. In the Portugal-Israel case, CO_2-equivalent emissions per kg were almost 6 times higher for live transport, with fuel consumption similarly elevated. Shorter journeys reduce stress, morbidity, and mortality, thereby mitigating risks of transmissible diseases and associated economic losses. Indeed, EFSA (2002) has noted that poor welfare and prolonged transport increase susceptibility to disease, highlighting the association between transport duration and animal health risk. This emphasises the One Health interface between animal, human, and environmental health. Transitioning to meat and carcasses trade represents a feasible alternative and solution. Regarding the ongoing case studies, preliminary results for the Ireland–Spain route suggest that shifting from live calf exports to domestic processing and meat export reduces emissions (33.7 vs 35.1 kg CO_2e/kg meat). While economic cost remains higher (€6.77/kg vs €10.31/kg), this gap is expected to narrow with refined feedlot and processing assumptions. Animal welfare outcomes strongly favour meat export, as live export entails prolonged transport, increased stress, multiple handling events, sea crossing, and substantial live weight loss. Emerging policy developments and broader sustainability constraints—including Ireland's nitrate derogation and herd intensity limits—further challenge the long-term viability of the current system. In this context, shorter transport distances and carcass-based trade models may reduce emissions, operational vulnerability and disease transmission risk. Overall, integrating economic modelling with welfare, environmental, and policy analysis demonstrates that the transition from live animal exports to meat and carcass trade could represent a feasible alternative under current market and regulatory assumptions.

Assessment of carcass and meat quality of crossbreeds of local breeds and commercial lines under organic feeding regime containing alfalfa

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Local chicken breeds are valuable genetic resources for sustainable poultry production. They are adaptive to local environments but low in production performance. Nowadays, organic poultry products are preferred by consumers due to food safety under environmentally friendly production practice. Crossing local breeds with commercial lines possessing improved production performance is one of the solutions for sustainable poultry production and for conservation of valuable genetic resources. Use of local breeds in organic farming will promote their potential for economic relevance. In organic farming, it is not allowed to use artificially synthesized growth promoters and amino acids. Alfalfa, a locally available feed, is rich in essential amino acids, and can be considered as a feasible organic feed source. The present study was aimed at determining carcass and meat quality of crossbreeds fed organic diets containing sieved alfalfa. Three German, local chicken breeds, Altsteirer (ALT), Ramelsloher (RAM), Bielefelder (BIE) were crossed with commercial lines, layers (White Rock, WR) and broilers (Ranger, RG). The birds were fed organic diets containing alfalfa meal (5%) until week 12. Two dietary feeding regimes: control diet (5% alfalfa) and trial diet (15% alfalfa) were used in week 13-14, and the alfalfa was increased to 20% in the trial diet in week 15-18. At week 14, 16 and 18, 10 birds per cross per diet (n=360) were slaughtered to determine carcass and meat quality parameters. Data were analyzed in R using parental lines, diets and age as factors fitted in a linear mixed model. It was hypothesized that crosses of commercial broilers will be better in carcass and meat quality under this organic feeding regime. Across all ages, carcass and meat quality parameters were mostly influenced by mother lines. RG crosses possessed higher ($p<0.01$) carcass, breast and thigh weight than WR crosses. In terms of meat quality, there were lower storage loss (SL), cooking loss (CL) and shear force (SF) in RG compared to WR crosses. Higher Inosine 5′-monophosphate (IMP) and creatine contents were investigated in RG crosses. The fatty acid contents (SFA, MUFA, PUFA) were higher ($p<0.01$) in WR crosses. Among father lines, carcass weight in weeks 14 and 18 were higher ($p<0.001$) in BIE crosses (1,089 and 1,616 g) compared to ALT (1,029 and 1,566 g) and RAM (1,006 and 1,520g). IMP of ALT crosses was lower ($p<0.001$) than BIE and RAM at week 14. Inosine of RAM crosses was higher ($p<0.05$) than BIE crosses while ALT crosses were similar to both at week 18. BIE crosses had higher ($p<0.05$) SFA than ALT at week 14 and higher ($p<0.05$) n-6 PUFA than RAM at week 18. Regarding the diet, there were no significant different effects of diets, except higher carcass weight in the control at weeks (16-18). In chemical composition, crosses in the trial diet were lower ($p<0.05$) in IMP and inosine at week 18, but higher in MUFA and n-3 PUFA across ages. According to age groups, crosses at week 16 had higher ($p<0.05$) in SL of breast, lower ($p<0.001$) CL of breast and thigh than those at week 18. Overall findings reflected the genetic potential of parental lines in crossbreeding. RG and BIE crosses demonstrated higher carcass and better meat quality. No significant effects of diets highlighted the contribution of dietary alfalfa meeting nutrient requirements of crossbreeds, except carcass weight at weeks 16-18. RG and BIE crosses have been shown to be most suitable for high-quality carcass production under organic feeding regimes.

The effect of broiler production system on carcass quality

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The Dutch broiler sector is rapidly transitioning toward production systems based on slow-growing genotypes. Despite their increasing market share, scientific knowledge specific to slow-growing broilers, particularly regarding carcass quality, remains limited. Most existing insights originate from studies on conventional fast-growing broilers, leaving key gaps in understanding resource efficiency, behaviour, health, environmental sensitivity, and slaughter outcomes in slower-growing broiler production systems. This study therefore evaluated the effects of season, catching method, and thinning on carcass quality and production parameters across four broiler production systems in the Netherlands. Slaughterhouse data collected from 2018 to 2020 included conventional fast-growing broilers (CONV; n=14,976 flocks), two indoor slow-growing broiler systems (SGB1; n=1,730 and SGB2; n=3,713), and the Better Life 1-Star broiler system (BLS; n=1,121). Mixed-model (REML) analysis was used to estimate the fixed effects of season (via Fourier transformation), production system, slaughter location, and catching method, as well as random coefficients for carcass quality traits. Across all slow-growing systems, slaughter weight and average daily gain (ADG), first-week mortality, and total mortality were consistently reduced than in CONV broilers. SGB1 showed higher ADG than SGB2 and BLS. Seasonal patterns were evident: slaughter weight and ADG were lowest for chicks placed in March/April and highest for those placed in September/October. All slow-growing systems (SGB1, SGB2, and BLS) demonstrated improved welfare and carcass quality outcomes, including reduced footpad lesions, hock burns, leg and breast hematomas, dead-on-arrival birds (DOAs), and total carcass rejects compared than CONV broilers. Season influenced lesion patterns, with autumn flocks showing more hock burns, footpad lesions, and wing hematomas than spring flocks. Summer flocks had more scabby hips but fewer total rejects relative to winter flocks. Thinning was associated with more scabby hips, ammonia burns, and DOAs, but fewer other lesion types and carcass rejects compared with non-thinning flocks. Mechanically caught flocks had more ammonia burns, DOAs, and total rejects than manually caught flocks. In conclusion, this large-scale analysis demonstrates that slow-growing broiler production systems consistently achieve lower mortality and improved welfare-related carcass quality indicators compared with conventional fast-growing systems. Seasonal and management factors significantly modulate these outcomes, underscoring the need for system-specific optimisation as the sector transitions toward slower-growing broiler production.

Genetic characterization of Extremeña Azul, an endangered native Spanish chicken breed, using 30 microsatellites markers

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Poultry biodiversity is considered one of the most endangered genetic resources considering the general loss of genetic variability in highly selected species. Commercialization of chicken breeding favored the use of highly productive chicken breeds and consequently led to lower population sizes of low performing native breeds. Aware of the importance of preserving native breeds, Regional Government of Extremadura launched a recovery program of Extremeña Azul breed based on a few existing animals which currently numbers around 2,300 individuals of 54 different breeders. Molecular markers are essential for genetic management to ensure high accuracy in breeding and conservation programs. Laboratorio Central de Veterinaria (Madrid) proposes two multiplex PCR to analyze the thirty microsatellite markers (ADL0112, ADL0268, ADL0278, LEI0094, LEI0166, LEI0192, LEI0234, MCW0014, MCW0016, MCW0020, MCW0034, MCW0037, MCW0067, MCW0069, MCW0078, MCW0080, MCW0081, MCW0098, MCW0103, MCW0104, MCW0111, MCW0123, MCW0165, MCW0183, MCW0206, MCW0216, MCW0222, MCW0248, MCW0295 and MCW0330) recommended by the joint ISAG/FAO Standing Committee for the genetic characterization of chicken breeds. Changes in primers were required to achieve a final configuration allowing all markers, in each PCR multiplex, to be analyzed together. Data from 246 samples analyzed show that proposed microsatellites were all well-coamplified in chicken blood samples and were all polymorphic, obtaining an average number of 4.97 alleles per marker (4.70 in males and 4.80 in females). Number of alleles ranged from three markers showing only two alleles (MCW0098, MCW0103 and MCW0248) to one marker with eleven alleles (MCW0104) and another with ten (LEI0234). Mean heterozygosity was 0.6089 and PEG greater than 99.9999%. Data obtained of microsatellite markers used in this study can help to manage breeding programs, as well as planning future crossing programs.

Assessing the feasibility of physical enrichments for guinea fowl (Numida meleagris): A combined behavioural and economic analysis

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In intensive poultry systems, lack of environmental stimuli can impair animal welfare. Environmental enrichment is a proposed mitigation strategy, but its effects in guinea fowl (Numida meleagris) remain unexplored. This study, part of European Partnership on Animal Health and Welfare (EUPAHW), aims to quantify guinea fowl interactions with enrichments under commercial conditions and assess economic implications. Before the case study, an extensive literature review (Web of Science) was carried out to assess existing experimental protocols and guide the study design. The search terms used were: (guinea fowls OR broiler) AND (enrichment) AND (animal welfare). A systematic literature search identified 2,310 records. After removing duplicates (n=279), records were excluded based on year (n=1,506), language (n=17), article type (n=126), and irrelevant outcomes/topics (n=622), yielding 39 eligible studies from databases. Manual reference screening added 8 studies, resulting in 47 studies included to inform the study design. The study analysed video footage collected during three production cycles (1–80 days) conducted in 2024 at a commercial guinea fowl farm in Italy, with 12,000 guinea fowls per cycle. In each cycle, enrichments (bamboo canes, coloured plastic bottles, egg trays, straw bales, and CDs) were placed in different areas of the pen. The ethogram includes pecking, rubbing, jumping/onto, and other contact. Through focal-animal sampling, the frequency and duration of behaviours were recorded continuously for 5 minutes at two timepoints for one day in weeks (AM/PM). Six cameras per cycle recorded focal pens, with details on placement, height, and filming schedule. From 447 recorded videos, 190 were selected for behavioural analysis; 257 were excluded due to poor quality or insufficient duration. Cycle 2 was entirely discarded as recordings lasted only a few seconds. Cycle 1 contributed 96 usable videos recorded from 24 May to 2 August, distributed across six focal pens with different enrichment combinations. Cycle 3 contributed 94 usable videos recorded from 12 September to 29 November, distributed across five focal pens with various enrichment combinations. The recorded videos will be analysed by the Behavioural Observation Research Interactive Software (BORIS, v 9.7.1). Housing and management data were collected, including stocking density, cycle duration, barn characteristics, and technical equipment. Production parameters such as mortality, live weight, feed conversion ratio, and water consumption were recorded, along with labour inputs and detailed economic costs (feed, health care, energy, litter, and enrichment). All data will be analysed to compare production costs per kg of meat between a baseline scenario and the enrichment scenario derived from the case study. This study presents a methodological framework for evaluating environmental enrichments in commercial guinea fowl farming. The literature review informed the development of the experimental protocol and ethogram. Work is currently in progress: video analysis using BORIS software and economic calculations are being carried out. Final results will provide insights into the welfare and economic implications of environmental enrichments for guinea fowl production systems. This work was co-funded by the European Union's Horizon Europe Project 101136346 EUPAHW. Views and opinions expressed are those of the author(s) only and do not necessarily reflect those of the European Union or the European Research Executive Agency. Neither the European Union nor the granting authority can be held responsible for them.

Feather pecking in laying hens: redirected foraging meets a neuroimmune–microbiome gut–brain feedback loop

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Feather pecking (FP) remains one of the most consequential welfare challenges in commercial pullets and laying hens worldwide. Uniquely, FP is both an indicator and a cause of compromised welfare and persists across housing systems. Its expression is context-dependent and intermittent, emerging in some individuals, flocks, or years but not others, consistent with genetically influenced yet not genetically fixed vulnerability and shaped by stress physiology, environment, and the microbiome. This talk integrates two historically separate perspectives into a single process. Ethological view: when hens lack access to the coarse, fibrous, manipulable substrates they are biologically motivated to forage for, this unmet need functions as a stressor. Birds forage further and redirect pecking toward feathers as a specific appetitive substrate, a pattern observed even with outdoor access when nutritional/sensory needs remain unmet. Ingested feathers act as a physiologically meaningful resource by increasing gut motility and mimicking some benefits of coarse fibre; they can also foster beneficial Lactobacillus communities, a clue to why foraging-rich environments may reduce FP risk. Dysfunctional view: repeated stressors, nutritional, social, early-life, or chronic, then alter gut microbial composition and metabolites, shifting neuroimmune–microbiome gut–brain signalling. FP phenotypes often show lower Lactobacillus abundance and dysmotility in the gut, and tryptophan-metabolic shifts. Targeted pre-and probiotc-based diets can modulate immune–metabolic crosstalk, increase circulating tryptophan, and alter FP and feather damage, indicating that microbial manipulation can modulate gut–brain communication in susceptible hens. This integrated framework links stressors to physiological, genetic, and behavioural factors that together shape welfare outcomes and potentially explain why FP flickers on and off across flocks and years. It also points to actionable prevention beyond beak trimming: (1) probiotic/prebiotic strategies to stabilize neuroimmune pathways; (2) foraging-rich diets and substrates; (3) early-life enrichment to build stress resilience and behavioural flexibility; (4) strain-aware selection and management to reduce susceptibility; and (5) integrated monitoring that pairs behaviour with microbiome, immune, and stress-physiology indicators. Feather pecking may start for different reasons, such as unmet foraging needs or exposure to other stressors, but it continues once stress disrupts the gut, the microbiome, the immune system, and the brain. These systems then reinforce each other in a loop that maintains the behaviour. Recognizing this loop potentially help us design more effective, long-lasting prevention strategies.

Keel bone damage of local German chicken breeds and their crosses with high performing laying hens

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Keel bone damage (KBD) is a major welfare problem in laying hens, affecting up to 90% of birds in commercial production systems and potentially causing pain and reduced mobility. Local chicken breeds are often assumed to be more robust than high-performing commercial layers with regard to their health, but empirical evidence supporting this assumption is limited. Within the ÖkoGen project, we aimed to develop strategies for preserving three local chicken breeds—Altsteirer (ALT), Bielefelder Kennhuhn (BIE), and Ramelsloher (RAM)—and evaluate them for their suitability in organic farming. Due to their low laying performance, crosses with high-performing White Rock (WR) hens were also produced. The aim of this study was to compare keel bone damages in purebred hens and in their crosses. Two longitudinal X-ray studies were conducted to assess KBD in purebred hens (28 BIE, 38 ALT, 45 RAM) and crossbred hens (50 each of BIE x WR, ALT x WR, and RAM x WR). Birds were housed in an aviary system from 18 to 72 WoA. Radiographs were taken repeatedly at certain ages (purebreds: 18, 34, 41, 50, 60 and 70 WoA; crosses: 18, 24, 35, 40, 50, 60 and 70 WoA). Individual laying performance was recorded daily throughout this period. Keel bones were divided into cranial, medial, and caudal sections and scored for fractures/calluses on a scale from 0 (no damage) to 3 (severe damage with multiple fractures and dislocations). These scores were combined into a single overall score per bird. KBD scores were analysed using a generalised mixed model based on a Poisson distribution, with observation time included as a polynomial covariate (up to the fourth degree) to account for non-linear age effects, and breed designated as a fixed effect. KBD scores increased significantly with age in both purebreds and crossbreds, with a more pronounced increase observed in ALT than in the other breeds. Among the purebreds, BIE hens showed significantly lower scores than ALT hens, while RAM hens had intermediate scores with no significant differences to BIE or ALT. In the crosses, BIE x WR had significantly lower scores than RAM x WR. RAM x WR hens showed a stronger increase in the KBD scores over time than the other crossbreds. Overall, KBD scores were higher in crossbred hens than in purebreds. In this context it should be noted that crossbred hens also exhibited substantially higher laying rates, nearly twice those of the purebred lines (72–78% vs. 30–39%). These findings indicate that genetic background and production level influence susceptibility to keel bone damage. The substantially higher laying rates in crosses may be associated with their increased KBD, suggesting that both breed and egg production contribute to keel bone health and welfare in laying hens. Our results suggest that local breeds, here Bielefelder Kennhuhn in particular, may improve keel bone stability in high performing crosses. Further analyses will investigate bone mineral density and blood parameters related to calcium homeostasis. The project was supported by funds of the Federal Ministry of Food and Agriculture (BMEL) based on a decision of the parliament of the Federal Republic of Germany via the Federal Office for Agriculture and Food (BLE) under the Federal Programme for Ecological Farming.

From performance to footprint: Zootechnical and environmental effects of a protected sodium butyrate supplement in broiler production

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The livestock sector is facing increasing societal and regulatory pressure to adopt more sustainable production practices. Within poultry production systems, environmental assessments consistently identify climate change, acidification, and eutrophication as the main impact categories. In this context, protected sodium butyrate (PSB) has gained attention as a functional ingredient with the potential to improve environmental performance. PSB supplementation has been shown to enhance nutrient digestibility, reduce litter moisture and nitrogen excretion, factors that are closely associated with lower ammonia emissions. Together, these improvements in nutrient utilization and performance position PSB as a promising strategy for reducing the environmental footprint of poultry production. This study aims to quantify the contribution of PSB to the environmental footprint of broiler production under Spanish conditions, using a life cycle assessment (LCA) approach with system boundaries defined up to the farm gate. Two comparable broiler production systems were therefore evaluated: a control system and a supplemented system incorporating PSB at a dietary inclusion level of 0.1%. Both LCAs were conducted in accordance with ISO 14040 and ISO 14044 standards and aligned with FAO guidelines for feed additives and poultry supply chains. A cradle-to-farm-gate system boundary was applied, and environmental impacts were assessed using the Environmental Footprint (EF) 3.1 method. Direct manure emissions were modelled following IPCC and EMEP methodologies. Nitrogen excretion was calculated based on measured feed intake, body weight gain, and feed composition. The functional unit was defined as 1 kg of live broiler at the farm gate. The Spanish broiler production system was modelled assuming a 42-days production cycle. Zootechnical experimental data were collected to contribute to the LCA calculations. 400 male broiler chickens were divided into 2 groups: control and PSB which only differed by the addition of the ingredient at 0.1% in feed. Performance (weight, feed intake) and mortality were measured at each stage and in the entire production period. Zootechnical results indicated that PSB supplementation significantly increased final body weight from 2.926 to 3.027 kg per bird (+3.45%, $p<0.05$), while feed consumption remained similar between groups. LCA results showed that the most pronounced relative reductions were observed for Acidification (−4.6%, from 1.51×10^2 to 1.44×10^2 mol H□ eq / kg live weight), Marine Eutrophication (−4.7%, from 6.64×10^2 to 6.33×10^2 kg N eq / kg live weight), Freshwater Ecotoxicity (−3.3%, from 64.01 to 61.91 CTUe / kg live weight), Total Climate Change (−3.1%, from 1.85 to 1.80 kg CO_2 eq / kg live weight), and Water Use (−3.1%, from 1.69 to 1.64 m^3 world eq / kg live weight). In addition, ammonia emissions were reduced by approximately 10.4%, decreasing from 0.000968 to 0.000867 kg NH□/kg live weight modelled mitigation effect. The productivity gains associated with PSB supplementation translated into consistent reductions in environmental impacts per kilogram of live weight produced, with an overall decrease of approximately 3% across impact categories. The reduction in ammonia emissions should therefore be interpreted as a modelled outcome of improved nitrogen utilization efficiency rather than a direct modification of emission factors. Overall, the combined zootechnical and LCA results indicate that PSB supplementation provides a dual benefit by enhancing broiler productivity while simultaneously reducing environmental burdens per unit of live weight produced.

An innovative plant-based solution that helps prevent the negative effect of coccidiosis

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Coccidiosis remains a major challenge in the poultry industry, significantly impacting the health and productivity of broiler chickens worldwide. This parasitic disease, caused by species of Eimeria, leads to considerable economic losses due to impaired growth performance, intestinal damage, and increased mortality. It also increases reliance on anticoccidial drugs. Developing nutritional solutions for broiler chicken is a promising approach to improve their overall health and resilience against diseases like coccidiosis. The objective of this study was to assess the preventive efficacy of an innovative nutritional liquid solution on zootechnical performance and coccidiosis-related lesions in broilers under field challenge. A total of 450 male Ross308 broilers were randomly allocated to three groups on a commercial farm with a documented coccidiosis history: a negative control receiving a coccidiostat-free diet (CTRL–), a positive control receiving feed containing coccidiostats (CTRL+), and a treated group (TRT) receiving commercial feed without coccidiostat plus an innovative plant-based solution. This preventive solution was provided in the water, ad libitum, from day 1 to day 41. Birds were housed in pens of 15 (10 pens per group), individually identified, and monitored for body weight and average daily gain (ADG) on days 4, 11, 21, 28, 35, and 41. Coccidiosis-related lesion scoring was performed on three birds per pen (30 birds per group) on days 21 and 28. Total oocysts count was performed at the pen level on days 21 and 28. The statistical unit varies between individual bird and pens depending on the parameter being measured. Statistical analyses were conducted at the appropriate experimental unit using ANCOVA for ADG, ANOVA for body weight, and Kruskal–Wallis tests for lesion scores and oocysts counts (significance threshold $p < 0.05$). Both TRT and CTRL+ groups consistently exhibited higher body weights than CTRL– at day 21 (991 g / 993 g vs. 957 g, $p = 0.023$), day 28 (1602 g / 1588 g vs 1503 g, $p = 0.015$), day 35 (2356 g / 2348 g vs 2187 g, $p = 0.002$) and day 41 (3051 g / 3081 g vs 2920 g; $p = 0.049$). The TRT group demonstrated a significantly higher ADG between days 4 and 11 (34 g/day vs. 32 g/day for both controls, $p = 0.05$), indicating improved early growth dynamics. On day 21, the TRT group showed a significant reduction in lesions caused by Eimeria acervulina ($p = 0.026$), indicating targeted efficacy in the early stages of infection. Furthermore, lesion prevalence also differed significantly among treatments ($p = 0.040$), with 70% of TRT birds exhibiting a 0-score compared with 50% for CTRL+ and 40% for CTRL–. No significant differences were observed on day 28, likely reflecting the onset of adaptive immune responses across groups. Oocysts excretion was similar between groups on days 21 and 28. The innovative liquid nutritional solution demonstrated preventive efficacy by reducing early coccidiosis-related lesions and supporting improved growth performance under field challenge conditions. These results support the relevance of nutrition-based strategies as complementary tools to enhance broiler resilience and mitigate the impact of coccidiosis in commercial production systems.

Effects of slow-releasing calcium supplementation on growth performance, production performance, egg quality, nutrient digestibility, and blood profile in old laying hens fed a corn–soybean meal diet

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Calcium (Ca) is one of the most critical macro-minerals in poultry nutrition, playing a fundamental role in skeletal integrity, eggshell formation, and numerous metabolic processes. In laying hens, particularly those in the late laying period, adequate calcium supply is essential because birds must continuously mobilize Ca for eggshell mineralization while maintaining bone homeostasis. This study evaluated the effects of graded supplementation of slow-releasing calcium on performance, egg quality, nutrient digestibility, blood mineral and bone turnover responses in aged laying hens. A total of 336, 54-weeks-old Hy-Line brown laying hens were randomly allocated to four treatments (7 replications, 12 hens per cage) for 12 weeks. The dietary treatments were as follows: CON, corn–soybean meal–based basal diet; TRT1, CON+ 0.25% slow-releasing calcium (SRCa) ; TRT2, CON + 0.5% SRCa; TRT3, CON + 0.75% SRCa. Data was analyzed in a complete randomized block design using the GLM procedures of SAS (SAS Institute, Cary, NC, USA). Linear and Quadratic polynomial contrasts were used to examine the 0.25- 0.75 % of SRCa supplementation. $P < 0.05$ was set as significant. Production parameters were not affected by dietary treatment. The proportion of downgraded eggs did not differ during weeks 1–6 but was significantly reduced from weeks 7–12 ($P < 0.05$) in hens fed higher levels of SRCa. Egg quality parameters were not affected during weeks 1–6 except for eggshell thickness, which was improved ($P < 0.05$) by increasing levels of SRCa during week 6; however, from weeks 7–12, eggshell strength and eggshell thickness were significantly ($P < 0.05$) increased in hens fed the 0.75% SRCa diet compared with the CON and lower inclusion levels. Apparent digestibility of ash and phosphorus was not affected by dietary treatments, whereas calcium digestibility increased ($P<0.05$) linearly in hens fed the 0.75% SRCa diet. Blood calcium concentration was increased ($P<0.05$) linearly among treatments at all post-feeding sampling times. Concentrations of parathyroid hormone, osteocalcin, and C-telopeptide were significantly reduced ($P < 0.05$) at later post-feeding times in hens fed higher levels of SRCa. In summary, dietary SRCa up to 0.75% improved eggshell quality, enhanced calcium digestibility, increased calcium circulation, and reduced bone turnover in aged laying hens without adversely affecting performance.
Keywords: Calcium, egg production, laying hen, blood mineral.

Integrating life cycle assessment into broiler feed formulation: balancing environmental gains, performance, and welfare

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Feed production is the main contributor to the environmental footprint of broiler systems, accounting for 50–97% of the impacts associated with climate change, eutrophication, acidification, land occupation and non-renewable energy use. Life Cycle Assessment (LCA) is therefore a key tool for designing feeding strategies that reduce environmental pressures at the most influential stage of production. This study evaluates the potential of LCA-driven, multi-objective broiler feed formulation to reduce environmental impacts while keeping zootechnical performance comparable to a least-cost control diet. Environmental impact values of all raw materials were incorporated into formulation software using ECOALIM V9 data, enabling optimization on six LCA indicators: climate change, non-renewable energy use, eutrophication, acidification, land occupation and phosphorus consumption. Six diets were produced: a least-cost control; four single-indicator diets targeting 15–30% reductions in either climate change or non-renewable energy; and one multi-objective formulation constraining all indicators to limit burden-shifting. Full LCAs of final feeds, including premixes, verified that predicted reductions were achieved. All targeted reductions were confirmed. Environmental improvements were mainly driven by substituting imported soybean meal with locally sourced protein ingredients (faba bean, toasted European soy, rapeseed or sunflower meal) and adjusting cereal proportions (increasing wheat, reducing maize). As expected, these changes also generated environmental trade-offs. Single-indicator formulations led to marked increases in land occupation (up to +60%), reflecting the lower yields of European protein crops compared with Brazilian soybean production. By contrast, the multi-objective formulation showed a more balanced environmental profile, reducing five of the six indicators while limiting the increase in land occupation (+27%). Importantly, it was the only diet that reduced phosphorus consumption relative to the control. Zootechnical results showed that moderate LCA constraints (R2–R4) maintained growth and feed efficiency comparable to the control. In contrast, strong constraints on a single indicator (R5: –30% energy use) decreased final weight (–3.7%) and worsened feed conversion. The multi-objective diet (R6) also showed a slight FCR deterioration, partly due to higher fine particles and fat content. This indicates that very strong constraints on a single environmental indicator may compromise growth performance, whereas multi-indicator optimization strategies maintain performance while limiting burden-shifting. Interestingly, some LCA diets (R3, R5) improved litter quality and reduced footpad dermatitis, linked to a lower dietary electrolyte balance caused by reduced soybean meal, suggesting potential welfare co-benefits. This reinforces the relevance of holistic sustainability approaches. Economically, moderate LCA diets increased cost by +3–8%, while heavily constrained diets (R5, R6) increased cost by +11–13%, partly due to poorer feed conversion. This confirms that improving environmental performance through LCA has a financial cost, which must be weighed against sustainability objectives. Overall, these findings demonstrate that integrating LCA into feed formulation is a robust and operational approach for reducing the environmental footprint of broiler diets while preserving performance relative to a conventional least-cost formulation. Future work will extend the LCA boundary to the farm-gate broiler product to quantify the system-level environmental benefits of implementing LCA-driven feed formulation strategies.

Towards sustainable pig farming in Sardinia: environmental impact and mitigation strategies

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The present study aimed at evaluating the environmental impact of Sardinian light pig production by a Carbon Footprint (CFP) approach. A cradle to farm gate CFP was performed in one intensive pig farm located in Southern Sardinia (Italy). Data on on-farm activities, inputs and output were collected through farmer interview, following the UNI EN ISO 14067:2018 standard. The temporal boundary was defined from 2019 to 2022, while the functional unit (FU) was 1 kg of live weight (LW) sold. Soil C sequestration was estimated by considering the above- and below-ground biomass residues, and the organic C from slurry deposition. The replacement of conventional soy with deforestation-free soy was evaluated as a potential mitigation scenario. Farm was 85 ha in size, raised 15,721±1081 pigs, and produced 2,145±82 tons of LW from light pigs. The average light pigs slaughter weight was 117.80±0.79 kg. Light pig production resulted in a climate change (CC) impact of 3.52, 3.64, 4.04, and 3.63 kg CO2e/kg LW sold for 2019, 2020, 2021, and 2022 years, respectively. Purchased feeds were identified as the main hotspot, accounting for 73.2% of the total environmental impact across all categories. The second largest emission source was from slurry management (9.9%). When soil C sequestration was included in the model, the CC lowered on average by 2%. Replacing conventional soy with deforestation-free soy reduced CC by 16%, while emissions from purchased feeds decreased by 22%. In conclusion, the production of light pigs appears to generate fewer emissions per FU compared to the production of heavier pigs, with purchased feeds being the main environmental driver. Therefore, replacing conventional soy with deforestation-free soy could be an effective mitigation strategy to reduce the CFP of Sardinian light pig production. The authors acknowledge the University of Sassari for the financial support (Mobilità Giovani Ricercatori, annualità 2024-2025; D.R. rep. n. 4422/2025, prot. n. 148562 del 23/12/2025).

Same name, same effect? Comparative in vitro repellent activity of Cymbopogon sp. essential oils in blends against poultry red mite

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It has been described that the extensive use of synthetic molecules treatment to control parasites can lead to the emergence of resistant species. Integrated control of parasites is nowadays a necessity for a resilient layer farming industry, to preserve the efficacy of chemical treatments. Plant extracts, and more specifically essential oils, are complementary tools that are being studied for poultry red mite (Dermanyssus gallinae) (PRM) management. Previous studies demonstrated the repulsive effect of Cymbopogon nardus, but the possibility of another Cymbopogon species with a different chemical profile, like Cymbopogon flexuosus, showing the same effect was not assessed. This study examined the effects of two blends of essential oils, primarily composed of essential oils from 2 different Cymbopogon species, on PRM in vitro. A two-choice behavioural assay was performed in Petri dishes. One side contained filter paper treated with a diluted blend of essential oil (10% in ethanol), while the opposite side contained ethanol only as an internal control. The first blend (S1) is mainly composed of Cymbopogon nardus, with citronellal and eugenol as the main active compounds. The second blend (S2) is mainly composed of Cymbopogon flexuosus with neral and geranial as the main active compounds. Clean empty dishes were used as external controls, with zones randomly assigned. PRM were released in the center of the dishes, and their distribution between treated and control areas was recorded after 20 minutes. Each treatment was replicated at least 10 times. Statistical analysis was performed using a generalized linear model (GLM) with a quasibinomial distribution, and Tukey test was used as a post hoc test. A total of 1230 PRM were used for this trial, 370 for S1, 365 for S2, and 495 for the control trial. Both essential oils significantly reduced the proportion of mites in the treated area (p-value <0.05). No difference was found between the areas of control Petri dishes. In the control petri dishes, the treated area contained, on average, 56% (+/-11%) of the PRM, while in S1 and S2, the + area contained, on average, 15% (+/-10%) and 19% (+/-16%) of the PRM, respectively. No significant difference (p-value = 0.64) was found between S1 and S2. A blend of essential oils composed mainly of Cymbopogon nardus and Cymbopogon flexuosus, even with different active compounds, has a significant repellent effect on PRM in vitro. Those results may indicate that both Cymbopogon species produce secondary metabolites with similar activity regarding PRM, or that the repulsive effect is barred or supported by minor compounds or interactions found in both essential oils. This question would need to be answered by other studies.

Performance stability of local and crossbred chickens under Ascaridia galli infection

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Local chicken breeds are frequently characterized as resilient to infectious disease and environmental stressors; however, robust quantitative evidence from controlled studies remains limited. We hypothesized that they exhibit high phenotypic resilience to Ascaridia galli (A. galli) infections, defined as minimal deviation in performance traits from matched uninfected controls, and that this resilience is retained in crossbreds. Four independent experiments used three local purebred genotypes (Altsteirer, Bielefelder and Ramelsloher) and their crossbreds with White Rock (ALT x WR, BIE x WR, RAM x WR). Growth performance was evaluated in purebred and crossbred male 'brother roosters', while laying performance and egg quality were analyzed in hens. Resilience was quantified through within-experiment, within-breed comparisons to contemporaneous uninfected controls. Birds were orally inoculated with ~1,000 A. galli eggs; controls received saline. A. galli-specific antibody titers were measured at 2 weeks post-infection (wpi) and at slaughter. Infection success (>70%) was confirmed by necropsy. Individual body weight, group laying rate, and egg quality traits were recorded for up to eight weeks. Statistical analyses were performed on absolute performance data; percent deviation from contemporaneous uninfected controls was calculated post hoc to describe resilience. Each experiment was analyzed separately. Data were evaluated using general linear models including genotype, infection status and where applicable their interactions. For repeated measurements, bird was included as a random effect; group effects were considered for laying data where appropriate. Non-significant higher-order interactions were excluded from final models. Estimated marginal means were compared using Tukey-adjusted post hoc tests, and false discovery rate (FDR) correction was applied where multiple comparisons were performed. Results are presented as mean ± SE, with significance set at $p < 0.05$. Across experiments, A. galli infection produced only minor phenotypic deviations from controls. Males of both purebred and crossbred groups showed a transient increase in A. galli-specific antibody titers at 2 weeks post-infection (wpi), with variation among breeds. Weight gain differed by ≤3 % from controls ($p > 0.05$). A significant Breed × Treatment interaction was detected in crossbred layers during the early infection phase (1–3 wpi). In RAM × WR layers, infection was associated with a marked transient reduction in laying rate (-34 % relative to controls, $p < 0.001$), coinciding with decreased shell breaking strength. Production subsequently recovered, to control levels by 6 wpi. No significant effects of A. galli infection on egg quality were observed. Crossbreds exhibited numerically lower within-breed variability in percent-change responses, suggesting more consistent performance under infection. Across experiments, A. galli infection had minimal impact on performance traits. Both local purebred and crossbred chickens maintained high phenotypic stability under infection. Crossbreds exhibited lower within-breed variability, suggesting that crossbreeding with a production-oriented line may support stable performance. These findings support the use of local breeds in sustainable, free-range poultry systems where performance consistency and welfare are priorities.

Dynamic changes in plasma triiodothyronine concentrations before and after introduction of newly hatched chicks

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Conventionally, after hens lay a clutch of eggs, they incubate the eggs and subsequently rear the chicks. This breeding cycle is referred to as broodiness. In modern laying hens, broodiness has largely been reduced or lost because of long-term selection for high egg production. However, understanding this inherent trait remains important for future breeding strategies and for maintaining genetic diversity of poultry strains. Prolactin (PRL), a hormone secreted by the anterior pituitary gland, plays a key role in maternal behavior and decreases markedly following chick introduction. The levels of other hormones also fluctuate throughout the reproductive cycle. Triiodothyronine (T3), is involved in metabolic regulation and has been reported to increase from the laying period through the incubation period and remain elevated during cohabitation with chicks. However, unlike PRL, detailed changes in T3 levels during the transitional phase associated with chick introduction remain poorly understood. In the present study, we investigated the plasma concentration of T3 before and after chick adoption. Six broody Gifu-jidori hens (a Japanese native breed) were housed individually under controlled temperature (20 ± 5°C) and photoperiod (14L:10D) conditions. On day 21 or 22 of incubation, immediately after blood sampling, five newly hatched chicks were introduced to each hen. The chicks were reared with the hen for 7 days (including the day of adoption) and then were separated. Blood samples were collected at consistent time points (17:00–18:00) on: (1) day 21 or 22 of incubation (pre-adoption), (2) days 1–3 post-adoption, (3) day 6 post-adoption (pre-separation), and (4) day 7 post-separation. Plasma T3 concentrations were measured in duplicate using a chicken-specific enzyme-linked immunosorbent assay kit. Statistical analyses were conducted using EZR, and comparisons were performed using paired t-test with a significance threshold of $P < 0.05$. Plasma T3 concentrations did not differ on days 1 and 2 post-adoption compared with pre-adoption levels; however, a significant increase was observed on day 3 post-adoption. In contrast, T3 concentrations significantly decreased 7 days after chick separation compared with pre-separation levels. Following chick adoption, a significant increase in T3 concentrations was observed in hens. This increase corresponded to the developmental stage at which chicks began to emerge from beneath the abdomens of the hen to seek food and water and to exhibit increased locomotor activity. During this stage, mother hens were required to recognize the presence of their chicks and maintain close proximity through vocalizations and movements, thereby guiding them to food and water. These findings indicate that T3 may be involved in maternal responses and behaviors in adoptive hens. Our results provide novel insights into the endocrine dynamics underlying maternal behavioral transitions in poultry.

Investigations into the farrowing process of organic sows in free farrowing systems

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A smooth and rapid farrowing process is a fundamental prerequisite for piglet health and vitality. Free farrowing systems offer distinct advantages, such as enhanced freedom of movement for the sows, but also present challenges, specifically limited opportunities for manual intervention during parturition.In 2025, 12 litters from F1 sows (German Landrace x German Large White) were monitored on an organic farm. The sows had a mean parity of 2.25. During farrowing, the duration of the birth process, inter-piglet intervals, umbilical cord condition, and the birth weights of live-born piglets were recorded and evaluated.All 12 farrowing processes proceeded spontaneously and without assistance. The average performance was 17.9 total piglets born (TB), 17.4 born alive (BA), and 10.8 piglets weaned. Gilts achieved a higher TB of 18.3 compared to 17.9 in older sows. These performance levels are categorized as highly positive. Despite the large litter sizes, the mean birth weight was 1.4 ± 0.25 kg, meeting the target weight for newborn piglets . The average farrowing duration was 245 ± 77 min, which is slightly shorter than in comparable studies. At 200 min, gilts were exactly 60 minutes faster than older sows (260 min). The mean inter-piglet interval was 14.3 ± 5.0 min. The longest intervals occurred between the 2nd and 3rd piglet (mean 29.25 min). Subsequent piglets were born at very short intervals, with only a slight increase to 17.25 min toward the end of parturition. Overall, expulsion times were brief: 69% of piglets were born within <15 min and 89% within <30 min, placing the majority of piglets within the optimal range. Only 5 out of 216 piglets had an interval exceeding 60 min, indicating a negligible incidence of dystocia. The sows utilized the freedom of the farrowing pens and moved during shorter breaks, which may have contributed to the low rate of birth interruptions. Regarding umbilical cord integrity, 91% of live-born piglets had intact cords at birth. Among the 6 stillborn piglets, only one cord was ruptured. This proportion of intact cords is remarkably high and exceeds findings in previous studies. The sows farrowed rapidly with short intervals and minimal complications. These results suggest that excellent farrowing outcomes can be achieved in free farrowing systems. Future research should investigate piglet mortality within the first three days of lactation, as this was beyond the scope of the present study.

Quality parameters of eggs from conventional and organic hens in mobile housing systems

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Egg freshness and shell quality are critical for the marketing of shell eggs. As the lay cycle progresses, egg weight increases, while hens face physiological challenges in providing sufficient calcium for shell deposition. Similarly, egg freshness tends to decline with both increasing hen age and prolonged storage duration. The present study evaluated hens of the genotypes Lohmann Tradition and ÖTZ Coffee. From the 20th to the 53rd week of life (WHL) in 2025, five randomly selected eggs per genotype were analyzed weekly. Parameters included egg weight, shell weight, shell thickness, egg length and width, albumen and yolk height, blood spots, shell thickness (measured at the sharp end, mid-section, and blunt end), and Haugh Units (HU). Starting at 49 WHL, eggs from both genotypes were subjected to three storage trials at defined temperatures (24°C, 22°C, and 20°C) for 21 days each. For these trials, 20 eggs per genotype were used, with five eggs analyzed per week. Data were processed using SPSS, employing univariate analysis of variance (ANOVA) and correlation analyses. The average egg weight was 64.8g±9.5, with a shell weight of 8.1g±1.1. Significant genetic differences occurred for both parameters: eggs from Lohmann Tradition were heavier (67.1g±10.0) than those from ÖTZ (62.6g±8.5). Shell weights differed between Lohmann (8.4g±1.1) and ÖTZ (7.8 g ± 0.9). No significant differences were found regarding egg length (58.1 vs. 58.7 mm) or width (44.8 vs. 43.2 mm). The incidence of blood spots was significantly higher in Lohmann Tradition eggs (1.1) compared to ÖTZ (0.4). While albumen height showed no significant variation (6.9 mm vs. 6.8 mm), yolk height differed significantly (17.8 mm vs. 18.6 mm). Eggshells were thickest at the sharp end (0.316mm±0.051), followed by the mid-section (0.316mm±0.053) and the blunt end (0.310mm ±0.054), with significant differences at each site. Eggs from ÖTZ hens generally exhibited thinner shells. The mean freshness (HU) was 79.3±14.2, with no significant difference between genotypes. However, the high standard deviation indicates substantial variability. Both genotypes showed a clear trend of declining freshness with age. During the first four weeks of lay, both genotypes exhibited excellent values (Lohmann: 92.8±5.0; ÖTZ: 90.5±5.2). By the 5th month of lay, values dropped to 78.1 (Lohmann) and 72.4 (ÖTZ), reaching very low levels by the 8th (68.9, Lohmann) and 9th month (62.8, ÖTZ). Accordingly, albumen height decreased from 8.4 to 5.7 mm (Lohmann) and 7.8 to 5.0 mm (ÖTZ). The correlation between HU and albumen height was 0.91, while the correlation between HU and egg weight was -0.42. These poor baseline HU values were confirmed in storage trials. Across all three trials, freshness decreased from a mean of 60.1 (fresh) to 16.3 (at 4 weeks), regardless of temperature. In the retail sector, a minimum of 65 HU is often specified. At the start of the storage trials, 50% of the tested eggs already fell below this threshold; by the end, 100% failed to meet this requirement. The precipitous drop to such low levels in these genotypes suggests that factors other than age must be considered. In conclusions, the egg quality of conventional and organic genotypes is comparable and generally maintains a high standard. However, the significant decline in HU values early in the laying period warrants further investigation into underlying causes.

Influence of a consistent arginine supply in turkey starter diets with varying protein contents

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Turkey diets are typically formulated with high crude protein (CP) and amino acid (AA) supply to support the high performance potential of modern genotypes. However, excess CP intake inevitably increases nitrogen excretion, contributing to environmental pollutions. Reducing dietary CP may not work effectively if one or more AA fall below requirements especially if requirements are not well estimated. One such AA is Arginine (Arg), which plays a role in multiple metabolic pathways, particularly as precursor of the signaling molecule nitric oxide. This study aims to demonstrate that a gradual reduction of CP in combination with a high Arg to Lys ratio will have no negative effect on growth performance of turkeys, but will lead to improvements in sustainability parameters, in particular to a reduced feed global warming potential (GWP). A total of 210 (B.U.T. Big 6) turkeys were housed in a trial facility and reared during the starter period (day 0 – 13). Pens (seven replicate pens per treatment holding 15 male birds each) were allocated to one of two treatments (T): a high standardized ileal digestible (SID) Arg to Lys ratio of 1.15 and either a commercial feed (T1: 26% CP) or a reduced CP level feed (T2: 25% CP). Diets were based on wheat, corn, soybean meal and sunflower meal, and were supplemented with crystalline Arg. Body weight (BW) was measured on days 0 and 13, feed intake (FI) determined for the period. Feed conversion ratio (FCR) and daily weight gain (DWG) were calculated accordingly. Additionally, the environmental impact of feed production was investigated through incorporating databases such as Global Feed LCA Institute (GFLI) enabling an accurate and customized environmental impact analyses. This study set the system boundary from cradle-to-feed gate following the PEF standard for feed producing animals. Performance data were statistically analysed using a one-factorial ANOVA with the fixed effect of treatment. Least square means were compared using Tukey test ($P<0.05$). Performance parameters were not affected by treatment. Final BW was numerically higher in the low CP group (T2: 396 g) than in the control (T1: 392 g), but both groups exceeded the breeder's performance objective (370 g). Feed intake (T1: 28.7 g/d, T2: 29.7 g/d), DWG (T1: 25.2 g/d, T2: 25.4 g/d) and FCR (T1: 1.14; T2: 1.17) remained with no difference between the treatment groups. The GWP of T1 was 1961 g CO2 equivalent per kg of feed while 1%-Point CP reduction (T2) reduced the GWP by about 14% to 1685 g CO2 equivalent per kg of feed. Turkeys maintain their performance despite a CP reduction in the starter phase if the AA requirements are fulfilled. The noteworthy environmental relief in the low CP group was mainly driven by the reduced soybean meal and soy oil as a result of low CP feed formulation. These results are in good agreement with a previous broiler study showing CP reduction with a consistent arginine supply (SID Arg to Lys ratio of 1.15) does not lead to a performance drop but improves sustainability parameters (Ullrich et al., 2018).

Impact of particle size and homogenisation on water populations measured by LF-NMR in poultry meat and its impact on water-to-protein ratio

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Commission Regulation (EC) No 543/2008 establishes maximum water-to-protein ratio limits in poultry meat cuts; however, not univocally described guidance on sample preparation has led to inter-laboratory variability in moisture determination. We hypothesised that particle size reduction and different homogenisation procedures modify water populations distribution in poultry samples, thereby affecting measured moisture content and water-to-protein ratio (Weesepoel et al., 2019). Low-field nuclear magnetic resonance (LF-NMR) was used to provide insight on the impact of sample preparation on water populations. Two independent sample sets were used. For particle size, six retail chicken breast samples were prepared under three conditions: A) 1 cm cubes; B) ground once; and C) ground twice (both 4 mm plate). For the homogenisation trial, 96 drumsticks were distributed into four processing groups: i) two-step process without liquid nitrogen (LN), minced through a 4-mm hole; ii) three-step process using LN (in contact with the material); iii) three-step process with LN but not in contact with the sample material; and iv) three-step process with storage at −80 °C. Water populations were quantified as bound (0.1–3 ms), immobilised (20–80 ms), and free water (200–1000 ms) according to relaxation times (T_2) using an Oxford MQC benchtop NMR (McDonnell et al., 2013). Moisture (ISO1442) and protein (ISO937) contents were determined. Data were analysed by ANOVA and correlation analysis ($P<0.05$). In fresh breast samples, particle size did not affect bound water but significantly altered the distribution between free and immobilised fractions. Reduction in particle size promoted and increased immobilized water while the opposite effect was seen in free water. Moisture content decreased significantly with decreasing particle size ($P=0.005$), with twice-ground samples showing lower values than cubes. After drying the fresh breast samplers, free water was fully removed and no significant differences in immobilized and bound populations were detected. However, samples with larger particle showed less consistency and a trend to increase the observed values of immobilized water. These results suggest an interaction between particle size and water populations. It has been described (Alvarez et al., 2023) how smaller particle size favors evaporation, and we observed how it leads to less free water available. For the second trial, using drumsticks, homogenisation significantly modified the proportions of free, immobilized, and bound water in homogenates. Free and immobilised fractions were strongly and inversely correlated ($r\approx1$), reflecting the very low proportion of bound water (≈0.001%). Both fractions were highly correlated with moisture content, protein content and water protein ratio ($r = 0.67$, 0.79 and 0.79, respectively), indicating that alterations in water mobility translate into analytical differences. These results demonstrate that mechanical disruption alters water distribution patterns consistent with structural modification of the sample matrix. Particle size reduction affects evaporation dynamics leading to changes in measured moisture content. Homogenisation significantly shifts water population profiles. The data confirm that sample preparation is a critical analytical variable. Standardisation of homogenisation procedures is therefore essential to improve reproducibility and regulatory compliance in poultry water determination.

Influence of mash feed particle size selection on feed intake patterns and performance in laying hens

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Modern birds are selected for their high feed intake to guarantee high performance. Feed is accurately formulated, but discrepancies in feed nutrient profiles can be observed between feed formulation, feed production and final feed intake. Mash feed is a challenging matrix due to particles heterogeneity, the technology of feed distribution and birds' behavior towards the selection of coarse particles first (ie. feed sorting). This study aimed at understanding the link between feed sorting and zootechnical performances. Feed intake of 52 Novogen layers of 42-week-old in individual cages was monitored during 1 week and zootechnical parameters were daily recorded during one month. A long day-length program (12–14 hours) was implemented to allow for a longer feed consumption time. Feed and feed refusals were sieved after one week, and particle size distribution was calculated. Feed sieve contained 4 consecutive sieves allowing the particles segregation in 5 categories: above 3mm, 2-3mm, 1-2mm, 0.5-1mm and below 0.5mm. Four feeding behavioral patterns were observed: 1/ for 83% of the hens, refusals contained only fines and average body gain during one month (BWGm) was +800g; 2/ for 8% of the hens, refusals were mainly composed of maize and BWG was +200g; 3/ for another 8%, refusals were dominantly soybean meal and BWGm was +900g; 4/ for 2% hens, refusals were carbonate coarse particles with an absence of BWGm. Particles size lower and higher than 2 mm were considered as fine and coarse particles, respectively. Laying performances were analyzed according to fines particles proportion in feed refusals (HIGH: more than 70%, MODERATE: between 57% and 70%, LOW: below 57%) and layer performance category (GOOD: > 5 eggs/wk, MIDDLE: 5 eggs/wk, POOR: ≤ 4 eggs/wk). Categorical data were analyzed by Khi2 test, while continuous data were analyzed by T-test to compare the 2 groups (SPSS Statistics 26.0 (IBM). Birds preferred coarse particles, eating first maize and soybean meal: the proportion of fine particles was 57% in feed and reached 70% in feed refusals. Heavier hens presented lower fines intake ($P<0.05$). As expected, POOR layers presented lower number of eggs laid per week, lower feed intake and downgraded feed conversion ratio ($P\leq0.001$). However, there was no significant difference in egg weight and hen body weight between good, middle and poor layers. In conclusion, this study demonstrates that feed sorting behavior in laying hens is highly heterogeneous and closely associated with differences in body weight gain and feed intake patterns. Indeed, nutritional imbalance due to selective feeding may affect reproductive performance before impacting body mass, highlighting the importance of feed particle size in mash feed.

Possible association between live weights and plumage or skin damages in laying hens

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Feather pecking and cannibalism are multifactorial problems affecting many laying hen herds. Which hens are predisposed to get pecked and suffer from feather and skin damage is still under investigation. This retrospective study aimed to examine whether the plumage condition of light or heavy hens differed from those within the target weight provided by the breeding company. Therefore, hens' live weight as well as plumage and skin condition were recorded and analysed for potential relations. Data from five different research projects conducted on health- and welfare-related problems of laying hens housed in different housing systems were included. In sum, data were collected from 5,764 animals from 17 different flocks of untrimmed laying hens (varying genetics) during several visits over their entire laying period. Moreover, variables regarding hens' housing condition were recorded. Furthermore, at each visit, a sample of hens (50-100) was weighed and scored for plumage und skin damages (Plumage score: 6-point scale; Skin score: 4-point scale). Subsequently, depending on each hens' weight deviation from the target weights provided by the breeding company (weight group with relation to the target weights = WTW), animals were grouped as "too light" with < -10% of target weight (L-hens), as "good" showing ±10% of target weight (G-hens) or "too heavy" with > +10% of target weight (H-hens). Using a general linear mixed model, potential influencing factors on the hens' plumage and skin condition were analysed. The results showed that most hens (74.9%) were categorised as G-hens (Mean 1,823 g) while L-hens made up 13.5% (Mean 1,569.9 g) and H-hens 11.6% (Mean 2,097.4 g). Hens from all flocks showed plumage damage and increasing maximum scores over the course of the laying period. Plumage damage in one body region that was rated at least with plumage score 1 occurred in 54.1% of the monitored hens. The statistical model on plumage damage revealed statistically significant effects of the laying phase, the season, the WTW and the colour of plumage, while the housing system showed no effect. Comparisons between seasons as well as between different phases of the laying period showed significant differences and the worsening in plumage condition over the course of the year. Furthermore, the plumage scores between WTW groups revealed significant differences; the highest maximum score was recorded for L-hens. Regarding the plumage colour, hens with brown plumage showed more and severe damages than those with white feathers. The statistical model on skin damages also revealed significant effects of the season as well as the laying phase, equal to the results of comparisons observed in plumage damages. As the weight classes with relation to the target weights (WTW) demonstrated an influence on the sum of plumage damage and the G-hens showed the lowest number of animals with plumage damage over the entire laying period, the results of this retrospective study highlight the importance of good weight management in laying hen husbandry. Given the increased problems documented for the lighter animals in the L-hen group, the question remains whether low body weights or plumage damage appeared first. Thus, future studies should focus on individual hens within a flock and follow their weight development as well as plumage condition.

Helminth infections in free-range laying hen flocks in Lower Saxony: status-quo, reduction strategies and evaluation of appropriate diagnostic methods

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With the increasing proportion of alternative housing systems, particularly free-range and organic production, gastrointestinal helminths such as Ascaridia galli and Heterakis gallinarum are gaining importance in laying hen health management. The endoparasite eggs are excreted via the feces, persist in the environment and accumulate in highly contaminated areas, especially in the vicinity of the housing facilities in free-range systems. Transmission occurs by ingestion of infective stages, directly from contaminated material or indirectly through intermediate hosts. Infections may cause clinical and subclinical impairment, including reduced nutrient absorption, decreased production performance and increased mortality. Chemical anthelmintics are commonly used to reduce helminth burdens; however, under EU-organic regulations (EU 2018/848), the application in organic production is restricted and may entail economic disadvantages. Consequently, effective alternative control strategies are increasingly required. To obtain a comprehensive overview of the current infection status of free-range laying hen flocks, to evaluate the efficacy of phytogenic and chemical anthelmintics as well as potential management strategies for reducing helminth pressure, a longitudinal field study is being conducted in 50 free-range laying hen flocks in Lower Saxony over one laying cycle. The study further aims to identify parasitological diagnostic methods that reliably reflect the flock-level helminth burden. To date, 19 conventional and 27 organic flocks were included in the study, with 32 stationary and 14 mobile housing systems. Each flock was visited three times during the production period (24, 50 and 80 weeks of age), while management and performance parameters were recorded systematically. In addition, fecal samples were collected every 10 weeks both before and after phytogenic or chemical anthelmintic treatment, to analyse and count the helminth eggs (Ascaridia galli, Heterakis gallinarum, Capillaria spp., Syngamus trachea, Trichostrongylus tenuis) per gram feces (via McMaster Method) at flock level. Additionally, deceased birds were subjected to necropsy to assess worm burdens on animal level. Preliminary results indicated that nearly 38 flocks showed no detectable helminth infections at placement (17-24 weeks of age). Infection levels increased steadily from approximately 40 weeks of age onward, with Ascaridia galli constituting the predominant species. Anthelmintic treatments applied from around 50 weeks of age reduced worm burdens, with chemical anthelmintics demonstrating the highest efficacy and, in treated flocks, reducing egg counts to nearly zero. The age-related increase highlights cumulative exposure risk in free-range systems and underscores the importance of continuous monitoring. The discrepancy between low infection levels at placement and subsequent increases indicated a key role of environmental contamination of outdoor areas in transmission dynamics. As the practical project phase is still ongoing, further analyses will clarify exposure-related transmission patterns and further evaluate the effectiveness of phytogenic anthelmintics. This project was funded as part of the EIP-Agri project Worm-free, “Status-quo of helminth burden in free-range laying hen flocks and development of a web-based application for risk assessment to improve animal health and optimize management”.

Comparative evaluation of housing systems on immunological, physiological, and productive performance in laying hens

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Maintaining physiological stability (homeostasis) is essential for animals' survival. Accordingly, any event that disrupts homeostasis constitutes stress, posing a threat to survival that requires substantial physiological resources to counter and restore stability. Restoration of homeostasis relies on the synergistic activity of multiple physiological systems, with the immune system playing a central and critical role. Animals raised under intensive farming conditions face numerous stressors. For several years, our laboratory has developed a minimally invasive method for measuring stress to provide reliable, quantitative markers of stress experienced by animals during rearing. This method addresses two key needs: first, improving animal welfare by identifying stressful management conditions; and second, detecting conditions that impair animal performance. To date, it has been applied to chickens (layers and broilers) and turkeys under various conditions, including stocking density, molting, feed withdrawal, transportation, and marketing. Here, using this methodology, we assessed stress status in commercial Lohmann laying hens under two housing systems (rafters' floor vs. community cages). One hundred layers were housed in four community cages, each containing 25 hens at 750 cm² per hen. The remaining 5,900 hens were kept in a free-range house with rafters' flooring at 9 hens/m², equipped with perches, nest boxes, treated drinking water lines, and feeders—all ad libitum. The experiment comprised three biological replicates. Each began with a 3-week recovery period, followed by group allocation, blood sampling after 3 weeks of treatment, and repetition of the sequence. Blood was sampled from 15 hens per treatment group per replicate. No differences were observed in physiological parameters such as hematocrit, total protein, or oxidative-active metabolites (AGEs) across groups. However, the rafters' floor group showed significant ($p<0.05$) increases in serum levels of antioxidant proteins (lysozyme and transferrin), the anti-stress hormone serotonin, anti-inflammatory cytokines (IL-10 and TGF-β), and serotonin receptor (HTR2a) gene expression in peripheral blood leukocytes (PBLs). Moreover, the rafters' floor group exhibited improvements in most productivity and egg white quality parameters, albeit at the expense of egg size and shell strength. These findings suggest that rafters' floor housing induces beneficial stress-reducing effects while having ambivalent impacts on productivity and egg quality.

Stopping detoeing in broiler breeder males is possible

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Toe clipping of males is a standard commercial procedure to prevent excessive damage to the hens. Although toe clipping in males may thus have a positive effect on female broiler breeder welfare, the procedure can cause stress, acute chronic pain and decreased leg health in males. The Dutch government wants to implement a ban on toe clipping before 2030. Therefore two on-farm studies were conducted on 19 breeder Ross 308 and 6 Hubbard JA57 farms, each including at least one house with non-toe clipped males. At the end of the rearing phase total male mortality was recorded. During the laying phase, 60 females per house were individual assessed at 28, 35 and 45 wk of age (woa). Feather cover of the females were scored on four different body parts (back, wings, tail, thighs) and skin damage on the thighs was recorded. Skin damage was also assessed at the slaughter plant. Production performance (egg production, mortality, male%, and fertility) were recorded by the farmer per house. Additional male management (lower male%, lower male BW up to 40 woa, lower light intensity) was applied to minimize the impact of males with untreated toes. Differences between houses with treated or untreated males were evaluated using paired-samples t-tests. Four out of nineteen Ross 308 flocks had an increased mortality due to thigh damage (>1%) around 30 woa. To prevent increased mortality and decreased welfare, males in these flocks were nail clipped at approximately 30 woa. During the rearing phase, mortality (including culling) among males was on average 7.5%-point lower in untreated males compared with treated males. However, differences between flocks varied widely (0% to >21%), largely due to reduced culling for leg health problems in untreated males. Average feather cover of the Ross 308 females did not differ at 28, 35 and 45 woa, although feather cover of the thighs of the females was poorer in flocks with untreated males at 35 woa. No differences on feather cover on the thigs was observed on 28 and 45 woa. No differences on feather cover and thighs skin damage was observed in the Hubbard breeders. On average, small skin damage on the female Ross 308 thighs were higher on 28, 35, and 45 woa. This higher incidence of skin damage was also observed in Hubbard JA57 flocks at 35 and 45 woa. Across all ages, average feather cover of females was poorer in houses where only the lower percentage of males was used. Additional management measures (lower BW of males and reduced light intensity) improved feather cover. Male to female ratio was on average 1.1% lower (7.6% vs. 8.7%) than standard practice. Female mortality at 30 and 60 woa, mortality due to thigh damage, number of total eggs, number of hatching eggs, and fertility did not differ between houses with treated or untreated males. In conclusion, applying additional male management strategies enables the discontinuation in the future of toe clipping in broiler breeder males.

Breeder farm environment and age as primary drivers of offspring performance and gut microbial succession in broiler chickens

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The maternal transfer of microbiota is fundamental to host-microbe interactions, yet it remains unclear how environmental stressors in breeder chickens influence the successional trajectory of offspring microbial communities. We utilised a large-scale commercial poultry model to investigate how contrasting parental habitats across key production phases, influence the transgenerational assembly of the gut microbiome. This study compared progeny from two breeder farms with matched genetics and ages but differing management and productivity levels (Farm A: high-performing; Farm B: low-performing). Breeder caecal droppings were collected at seven timepoints (13–57 weeks; P0–P7). Progenies were sourced from breeder flocks at pre-peak (P3), peak (P4), post-peak (P5), and decline (P6) phases. In each cycle, 4,920 Ross-308 chicks were assigned to 12 pens (410 birds/pen) and reared under commercial conditions. At day 40, performance metrics, including European Poultry Efficiency Factor (EPEF) and Feed Conversion Ratio (FCR), were recorded, and faecal samples were collected. Microbiome profiling was performed using Illumina sequencing of 16S and ITS rRNA amplicons. Data were analysed using generalised linear mixed models, QIIME2, and R (phyloseq, vegan, splinectomeR). Breeder diversity was significantly influenced by both farm environment and age ($R^2 = 0.51$). Farm A exhibited high early microbial richness that declined with age and higher global bacterial Shannon diversity ($p = 0.03$), whereas Farm B maintained more even but less dynamic communities. Occupancy-abundance analysis indicated greater homogeneity and a larger fraction of core rare taxa in Farm A (55.7% above neutral model vs. 37.6% in Farm B). Conversely, Farm B showed stronger niche restriction, with 15.7% of taxa falling below neutral expectations. Phylogenetic clustering also differed significantly at 33 weeks ($P < 0.05$), with Farm A showing higher overall bacterial net relatedness and nearest taxon indices. While both breeder farm and age also significantly impacted progeny performance ($p < 0.05$ for EPEF; $p < 0.01$ for 2-kg FCR), with offspring from younger flocks and Farm B generally performing better, progeny diversity was primarily governed by parental age ($R_2 = 8\%$). However, progeny from P4 Farm A breeders exhibited higher Chao1 diversity than those from Farm B. CODA-glmnet computational modelling demonstrated that parental microbial signatures could be predicted from progeny communities with high fidelity ($R_2 = 0.90\%$). We identified a conserved transgenerational ”microbial core”: comprising Odoribacter, Clostridium methylpentosum group, Kazachstania, and an unidentified fungal taxon, that persists across the host generation gap despite environmental variation. Source tracking revealed that the breeder microbiome at P1 (20 weeks) provides the greatest contribution to the progeny gut microbiome. These results suggest that while the host environment acts as a strong selective filter for the parental microbiome, a small but robust microbial lineage maintains vertical persistence. This lineage may mediate transgenerational phenotypic effects, highlighting parental health and age as primary drivers in the early-life assembly of the host-associated microbiome.

Feeding the future: Global challenges, risks, policy solutions and the role of livestock production

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Ensuring a balance between food supply and demand is essential for long-term food security. However, climate change and ecosystem degradation are placing increasing pressure on agriculture and food systems, reducing their resilience and contributing to more volatile yields. Additional pressures arise from geopolitical and global health crises, which disrupt supply chains and increase the volatility of input and output prices. While agricultural production is directly affected by these developments, it also contributes to the overshoot of planetary boundaries. This calls for measures to reduce the environmental impacts of food production. At the same time, feeding the global population with diets like those typically consumed in North America or Europe would require more agricultural land than is currently available. Therefore, shifts in consumption patterns are also needed to align food demand with supply. In order to build sustainable and resilient food systems and ensure long-term food security, a combination of short- and long-term measures is required. Whilst enhancing productivity and resource-use efficiency is widely regarded as one of the most effective short-term approaches to reducing negative environmental effects from agricultural production, achieving substantial and lasting emission reductions will also require long-term strategies that fundamentally transform agricultural production systems. To design effective and cost-efficient practices and policies that support this transformation, it is essential to consider farmers' abatement costs, and to assess and balance existing trade-offs at all levels, from farm to sector. Furthermore, a coordinated approach by all stakeholders is essential. A range of instruments, both taken by the government or private actors, can support farmers and consumers in their transition towards more sustainable food systems. Regulatory measures include restrictions on livestock stocking densities, nutrient surpluses, and greenhouse gas emissions in agricultural production, as well as restrictions on the sale or procurement of food products with high environmental impacts. Economic instruments can incentivize sustainable practices through agri-environmental payments, carbon pricing, taxes on emissions or nutrient losses, or subsidies for environmental-friendly technologies on the production side, as well as taxes on greenhouse gas-intensive foods on the consumption side. Nudges seek to influence individual behaviour without imposing restrictions on choices or substantially altering economic incentives. Examples include default enrollment in environmental programs and public recognition schemes for farmers, as well as default plant-based meal options, or menu redesigns for consumers. Information-based measures aim to improve decision-making by providing knowledge and transparency. These include agricultural extension services, advisory programs, training, and decision-support tools for producers, alongside environmental impact or carbon footprint labels for consumers. A policy toolkit that combines regulatory, economic, behavioral, and informational instruments is generally more effective than single measures in supporting the transformation towards more sustainable production and consumption patterns. Achieving sustainable and resilient food systems therefore requires coordinated transformations across production and consumption. Policy tools must be used to balance environmental, economic, and social objectives while accounting for stakeholder incentives and trade-offs. This will accelerate progress towards long-term food security and planetary health.

Navigating the perfect storm: Between Green Deal ambitions, CAP reform, and market realities

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Relevance. European livestock farming faces an increasingly contested operating environment. Animal scientists are asked not only to improve productivity, efficiency and animal health, but also to contribute solutions to challenges arising from climate policy, environmental regulation, animal welfare expectations and evolving consumer preferences. Understanding the economic, policy and market forces influencing the future of livestock systems is essential for researchers seeking to ensure that scientific advances remain relevant and applicable in practice. Background/Objectives. For many decades, European livestock farming operated within a relatively stable policy environment characterised by substantial public support under the Common Agricultural Policy (CAP), predictable regulatory frameworks, and expanding international trade opportunities. That environment is changing. Livestock producers are operating within a more uncertain economic and policy environment, shaped by evolving CAP priorities, changing regulatory frameworks, and increasing uncertainty in international trade policy. This talk examines the major economic and policy challenges confronting European livestock farming and explores their implications for livestock systems and animal science research. Discussion. European livestock farming is facing a perfect storm created by the interaction of policy, environmental and market pressures. First, many grazing livestock systems remain highly dependent on public support payments for their economic viability. Ongoing CAP reform raises important questions regarding the future scale, distribution and justification of this support, particularly in marginal and grassland-based production regions. Second, climate and environmental policy have become a central driver of change. Livestock methane emissions have moved to the forefront of policy debates, creating growing pressure for measurable emissions reductions. There is growing scientific evidence that some European regions have livestock densities that exceed environmental carrying capacity. In these hotspot regions, technological improvements alone may not be sufficient to achieve environmental objectives, and reductions in livestock numbers are increasingly likely to form part of future policy responses. Third, livestock sectors are increasingly exposed to global market and supply chain risks. Geopolitical tensions, supply chain disruptions and evolving trade agreements, including those involving Mercosur and Australia, create both opportunities and vulnerabilities. The pressure on European livestock producers to meet higher environmental and animal welfare standards raises questions about how competitiveness can be maintained in increasingly open markets where competing producers may not be subject to equivalent requirements. Future competition from alternative protein products may emerge in some market segments. Fourth, animal welfare is becoming an increasingly important component of both public policy and market differentiation. The future role of CAP support, regulatory standards and market-based incentives in promoting improved welfare outcomes remains an important area of debate. Conclusion. The future of European livestock farming will be shaped not only by advances in animal science but also by broader economic, political and societal developments. The social licence for animal agriculture will be retained not only by demonstrating its contribution to food security, rural livelihoods, environmental stewardship and animal welfare, but also by how it responds to societal concerns. How livestock systems adapt to these challenges, and how animal science contributes to that adaptation, will play a critical role in determining the future place of livestock farming within European agriculture and society.

Unpacking the promises of technology in farming: The economic challenge

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In the past decades, many authors have described the promises of the use of technology, especially precision livestock farming (PLF). However, although not many scientific studies are available, it seems that adoption of PLF technology lags behind. This despite a large number of larger or smaller research projects and many scientific publications. One of the elements that deserves our interest is the aspect of value of technology. Although technologies ensure various dimensions of more precise information, it is important to assess the value of the added precision of information. The value of information from PLF technology can only be unpacked if decisions that are taken, based upon the technology are (economically) better than decisions taken without this technology. Although PLF technology is aimed at farm animals, the potential use of information can be upstream or downstream from animal production. For instance in monitoring animal welfare, phenotyping potential breeding traits or monitoring environmental aspects such as NH3 emissions. All the value propositions of PLF technology are based upon the assumption that farmers will invest in this technology. However, it seems that the adoption of PLF technology has stagnated in recent years. A recent literature review aimed at PLF technology for improved health, showed that in 132 peer reviewed articles, published in the last 10 years, only 3 evaluated the use of the technology in decision making. All others were mainly aimed at algorithm development. These results more or less confirm the disappointing development of adoption of PLF. Research is still focused on detection algorithms, while in the evolution of research one would expect more work on the integration and automation levels. One of the approaches to unpack the potential of PLF technology is to change the perspective of research and application. Most research up to date is aimed at the development of PLF systems to support more or less traditional management. For example, almost all research aimed at improved mastitis management using PLF technology, is aimed at detecting clinical mastitis. However, reasoning from the potential of PLF technology in mastitis management, a number of other managerial areas could be determined. Which means that the sensors can be used in much more than one way. A second example that we studied lies in the detection of suboptimal mobility. Research typically is aimed at detecting lame cows, for curative trimming. By simulation modelling we showed that with changes in hoof care management enables a cost-effective use of PLF technology that also resulted in improved dairy cow welfare. Similarly we are working on the potential of sensors in poultry farming, looking at the potential value of PLF technology to improve management of coccidiosis. A final example of the need for creating value on the use of technology is in the development of sensors to monitor environmentally harmful gasses such as ammonia (NH3) in dairy barns. Such systems may be immensely important to reduce the environmental footprint of production. However, without a decent value proposition, they will not be used, leaving their potential unpacked. In conclusion (PLF) technology provides a lot of promises to improve the quality of animal production. To unpack these, future research must integrate technology with clear management and show the (economic) value of the use of these technologies. Simulation modelling is a useful tool to embed (economic) impact evaluation alongside sensitivity and specificity metrics and determine the tradeoffs between them. Addressing these technical, integration, and economic challenges is essential before technologies can contribute to value-driven animal management on commercial farms.

Beyond pure productivity: The strategic role of genetics, resilience, and health for business continuity

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Before the 1940s, animal breeding primarily entailed pure breeding, and breeding objectives for livestock were predominantly visual, toward an "ideal type" for the breed. Subsequently, breeding evolved from objectives of appearance to objectives involving performance. For example, the primary aim in broiler and pig production is rapid growth, superior feed efficiency, and high lean tissue growth rate. When it became recognized that animals selected for high production efficiency are more at risk for behavioral, physiological and immunological problems, the concept of sustainability has come to play a prominent role in agricultural production, resulting in broader defined breeding goals that include not only production traits but also functional traits, such as longevity and health. Livestock animals are required to perform in a wide variety of environmental conditions regarding climate and disease pressure, therefore, animals need to be selected that have the ability to combine a high production potential with resilience to stressors, allowing for unproblematic expression of a high production potential in a wide variety of environmental conditions. However, there is a lack of clearly defined indicator traits of resilience, which yet have to be included in standard breeding programs. Novel phenotypes of resilience can be based, for example, on fluctuations in production traits over time. Selection strategies for sustainable production systems can support high input–high output systems (sustainable intensification), where animals are selected for maximized production efficiency in environments controlled by precision livestock farming techniques. Alternatively, breeding strategies can support reduced input–reduced output systems based on selecting animals that are more robust to climate change and are better adapted to transform low quality feed (local feeds, feedstuff co-products, food waste) into meat. The latter scenario may result in reduced predicted yields, reduced production efficiency and possibly increased costs to the consumer. In particular, autochthonous breeds produced in and adapted to the local environments may harbor the genes necessary to adapt to suboptimal environmental conditions; marketing initiatives like the Spanish "100% Autochthonous Breed" label may help promote and conserve these breeds through market differentiation of their products. Genetic selection can also be applied to optimize other links in circular agricultural systems. For example, besides genetic improvement of livestock breeds (genotypes better adapted to the impacts of climate change, performing well on locally sourced, feed ingredients of variable quality), selection may be applied to local feed crops (enhancement of yield, protein content, stress tolerance, and attractiveness to pollinators, and reduction of anti-nutritional factors) and crop pollinators (improvement of pollination efficiency, foraging behaviors, or the capacity to vector biocontrol agents). In addition, animal breeding practices have become part of the debate that deals with issues of animal welfare and animal production ethics, where animal breeding goals may be broadened further to include emotional and societal values, such as animal well-being. It is the aim of the present perspective to present an overview of sustainable breeding goals that balance production potential with resilience and environmental sensitivity, increasing or restoring the animals' ability to interact successfully with the environment and improve welfare and productivity. For the successful implementation of sustainable production systems, cooperation is needed regarding all multidisciplinary actors involved.

The other side of a Curriculum Vitae: A case study in animal science

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A curriculum vitae records what a scientific career produces. It does not record how it actually works. Research careers in animal science are rarely the product of strategic planning. More often they emerge from a sequence of methodological investments, external constraints, and network effects that are only retrospectively legible as a coherent trajectory. This lecture uses one 35-year publication record in pig nutrition and meat science as primary evidence, chosen not because the career is exceptional, but because it is the only one for which all details, including the unflattering ones, are fully accessible. The record, spanning lipid metabolism, conjugated linoleic acids, pork quality and proteolysis, intrauterine growth restriction and foetal programming, boar taint, tannins as bioactive feed additives, protein and nitrogen efficiency, and former food products, is analysed not as a list of achievements but as a dynamic system driven by four identifiable forces: exhaustion of ideas, loss of financing, limits of personal knowledge, and dissolution of collaborating networks. A fifth force, pure curiosity meeting a prepared toolbox at a conference session, explains at least one major redirection that none of the other four can account for. A central argument is that methodological investments, once made, produce scientific returns over timescales that greatly exceed any single project or funding cycle. The corollary is that the value of a formative research period cannot be assessed from its immediate output alone. A second argument concerns what the lecture calls side effects: the committee memberships, editorial appointments, policy engagements, COST actions, and European research projects that accumulate alongside a publication record and that frequently prove more consequential than the publications themselves. These are not outputs of a career plan. They are consequences of sustained presence in a scientific community, built at conferences, in working groups, and over too many drinks considerably later than was advisable. The other side of a curriculum vitae is where a scientific career actually happens. So much for the past. What about the future? Animal science in 2040 will still be driven by the same fundamental curiosity, understanding the relationship between what an animal eats and what it becomes. But the questions that curiosity generates will be shaped by forces that do not yet have names. That is not a problem. Artificial intelligence, precision phenotyping, and multi-omics are not a revolution in what we want to know, they are a new toolbox, arriving faster than any previous one. These tools will reward the scientist who knows what question to bring to them. They will be less useful to the scientist who mistakes the tool for the question. Curiosity built this career. It will build the next ones too.

Economics of Genomic Improvement: How to Enhance Productivity While Reducing Environmental Impact

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Genetic improvement has long been a key driver of productivity in livestock systems. Today, it is increasingly recognised as one of the most impactful and cost-effective approaches to simultaneously improve productivity and reduce the environmental footprint of animal production. However, despite strong evidence of its benefits, the economic value of animal breeding is still not fully recognised across the value chain and is sometimes underestimated in sustainability debates. Advances in breeding technologies—supported by improved data availability, enhanced trait recording, and more precise selection methods—enable breeders to target traits that improve resource efficiency, robustness, health, longevity, and emissions intensity. Yet adoption remains unbalanced across species, production systems, and regions, reflecting differences in data infrastructure, investment capacity, market incentives, and policy support. There is therefore a clear need to reaffirm the role of animal breeding within the three pillars of sustainability: economic viability, environmental performance, and societal benefit. This FABRE TP Challenge session at EAAP 2026 will explore how investments in modern breeding approaches can deliver measurable gains for productivity while reducing environmental impact, and which economic and policy frameworks are needed to ensure that these benefits effectively reach breeding programs, farmers, and society. The session will examine how improved recording systems and phenotyping technologies provide the foundation for selecting animals that produce more with fewer inputs, lowering production costs and environmental burdens per unit of output. Speakers from academia, breeding organisations, industry, and agricultural economics—covering multiple species and production systems—will address key questions such as: What is the economic value of breeding, and how can this value be demonstrated across species?How do improved phenotyping systems, data infrastructures, and selection tools influence costs, returns, and risk along the value chain?How can productivity gains and environmental improvements be robustly measured, compared, and monetarized?What incentives, collaborations, and governance structures are needed to ensure that farmers and other actors benefit from upstream genetic progress?In line with FABRE TP's mission, the session emphasizes responsible, science-based breeding, strong cooperation between research and industry, and policy frameworks that recognise the contribution of genetic improvement to EU climate, food security, and sustainability objectives. A series of short expert presentations will be followed by an extended roundtable discussion, actively engaging the audience in identifying practical challenges, cross-sectoral solutions, and pathways for wider uptake. In conclusion, the 2026 FABRE TP Challenge aims to provide participants with a clear understanding of how economically viable breeding innovation can simultaneously enhance productivity and environmental performance, and how coordinated action across research, industry, policy, and practice can unlock its full potential across Europe's diverse livestock systems.

What kind of breeding for a sustainable livestock production worldwide? global priorities, constraints, and pathways forward

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As livestock production systems worldwide face mounting socio-economic and environmental pressures, breeding strategies must increasingly navigate complex trade-offs between productivity, resilience, and environmental impact. Advances in genomics and genetic evaluation are creating new opportunities to select animals with lower methane emissions without compromising productivity. Evidence shows that methane-related traits are heritable and can be integrated into multi-trait selection indices. Drawing on preliminary findings from the Third Report on the State of the World's Animal Genetic Resources, this contribution explores emerging global priorities, recent improvements, persistent gaps, and potential solutions for more sustainable breeding approaches. The diversity of production environments and stakeholder needs underscores that no single strategy can fit all contexts. Instead, breeding decisions must be framed holistically, integrating genetic, economic, and ecological dimensions, while also recognising the practical limitations imposed by field conditions, infrastructure, and capacity constraints that shape what is feasible in breeding programmes on the ground. Investment in research, phenotyping and breeding programmes will therefore be essential to support climate-smart livestock development and align genetic progress with mitigation goals.

Challenges in breeding for reduced environmental impact and other eco-system services

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Breeding objectives have shifted from initially focusing on productivity to profitability by including more traits related to efficiency, health, and fertility. Ecosystem services, such as reduced environmental impact, have largely not been valued and thus not been thoroughly considered in defining breeding objectives. The possibilities and challenges of including environmental impact in defining breeding objectives are highlighted, with a focus on the value chain of improved genetics vs the value chain for animal products.

Unlocking genetic progress: importance of data-driven selection to enhance productivity, economy and environmental impact

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Genetic improvement in dairy cattle creates cumulative and long-term value, but its economic and environmental benefits are not always measured consistently across the value chain. This presentation explores how breeding progress translates into farm-level profitability, improved production efficiency, and reduced environmental intensity. Genetic gain can be expressed through higher milk and solids output, lower feed and health costs, and greater resilience, with impacts measurable in €/cow/year and over longer-term sector performance. The presentation also highlights the importance of phenotyping, genomic selection, and data infrastructure in increasing selection accuracy and accelerating genetic progress. Better phenotypes for traits such as health, resilience, and methane proxies, combined with interoperable data systems and robust governance, improve both returns and risk management. Environmental outcomes can be assessed through emissions intensity and life cycle assessment approaches, while monetization frameworks support valuation of productivity and carbon-related gains. Finally, the presentation discusses incentives, collaboration, and governance mechanisms needed to ensure fair benefit-sharing among farmers, breeding organizations, processors, and society. Transparent data rights, co-funding models, and outcome-based approaches are essential to capture the full value of breeding innovation.

Balancing profit, greenhouse gas emissions and resilience in dairy cattle breeding using integrated modelling approaches

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Genetic improvement offers a cumulative opportunity to improve both productivity and environmental sustainability of livestock systems. While substantial research has focused on phenotyping individual greenhouse gas (GHG) emissions to enable direct selection for lower-emitting animals, environmental impacts also depend on herd dynamics, production efficiency, and resource use across the production chain. System-level indicators such as GHG emission intensity (EI) therefore provide a complementary perspective for linking genetic improvement with environmental outcomes. Moreover, EI indicators can be used to derive emission intensity values (IVs) of traits, analogous to economic values in breeding indices, enabling environmental objectives to be explicitly considered in breeding programmes. Meanwhile, increasing climatic variability highlights the importance of selecting animals that are resilient to environmental perturbations. However, the combined implications of EI indicators and resilience-oriented breeding objectives remain insufficiently investigated. In the recent studies, an integrated modelling framework linking environmental assessment and genetic simulation was developed. First, a herd-level bio-economic model coupled with life-cycle assessment (LCA) was used to estimate EI of dairy production and to derive IVs for key traits by quantifying the marginal impact of genetic changes on emissions along the production chain. These IVs were used to explore breeding indices balancing farm profitability and EI. Building on this framework, long-term breeding scenarios under climate change were simulated for alternative objectives prioritising either milk production or resilience traits. Phenotypes were simulated using mechanistic models of dairy cow physiology and were translated into herd dynamics, allowing the economic and environmental consequences of breeding objectives to be evaluated. The analysis showed substantial variation in the contribution of traits to both farm profitability and EI. Derived IVs revealed potential trade-offs between economic and environmental outcomes when constructing breeding indices. Simulated resilience-oriented breeding resulted in cows producing less milk per day but with earlier conception, lower dry matter intake, extended productive lifespans, and improved ability to cope with short-term perturbations. These changes improved the cow's lifetime profitability and reduced EI, despite a reduction in annual profit per cow compared with the production-oriented breeding objective. Collectively, these findings illustrate how IVs can complement traditional economic values to integrate environmental considerations into breeding indices while highlighting the potential long-term benefits of selecting for resilience in dairy cattle populations. Linking breeding simulations, mechanistic modelling, and LCA provides a comprehensive framework to evaluate sustainable breeding objectives. Such approaches could contribute to the design of breeding objectives that better balance profitability, resilience, and GHG mitigation in dairy production systems.

Investments in modern genomics and digital phenotyping to improve the economics of livestock production systems

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The global population is expected to exceed 9 billion by 2050, requiring substantial improvements in the efficiency of food production systems to meet rising demand. Livestock production will play a critical role in supplying high-quality protein, making continued refinement of breeding and production systems essential for both nutritional security and economic viability. Selective animal breeding has already delivered major gains in productivity and efficiency, with direct economic benefits for farmers. For example, egg production in laying hens has increased by more than 27% since the 1960s through genetic improvement, enabling higher output per animal, lower resource use per unit of product, and more predictable performance. These improvements translate into reduced production costs, improved margins, and greater resilience to market volatility at farm level. Conventional breeding approaches, however, rely heavily on phenotypic data that can be costly to collect or may raise animal welfare concerns. Investment in digital phenotyping technologies—such as computerized tomography image analysis and camera-based locomotion modeling—allows performance data to be collected with minimal animal disturbance and reduced labor input. This accelerates genetic progress while lowering operational burdens for farmers, supporting economically sustainable production systems. At the same time, not all production contexts are suited to intensive systems. Breeding strategies must also support cage-free systems and smallholder farming, where livelihoods and local economies depend on robust, adaptable animals. Innovations such as DNA-based tracking and egg printing technologies enable performance monitoring in cage-free laying hen systems, supporting regulatory compliance without compromising farm income. In parallel, programs focused on providing locally adapted poultry lines to rural farmers in Africa demonstrate how breeding can strengthen household incomes, support profitable value chains, and improve access to animal protein. Addressing the global food challenge will require steady, incremental improvements in breeding and technology that deliver higher efficiency, improved animal welfare, and tangible economic benefits for farmers across diverse production systems.

European integration of phenotyping, genomic and data research facilities to foster sustainable animal production and aquaculture

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European research in farm animals is mostly organized at a country and even at an institute level whereas the challenges faced by farm animal research cross borders. Additionally, the increasing needs for detailed phenotyping of animals in various environments question the adequacy between farm animal research capabilities and the questions initiated by the agroecological transition and the increasing society concerns about animal welfare and ethics in science. The desire to describe the components of overall characteristics more precisely requires measurements, samples and data that should be easily obtained from a large number of individuals, using procedures that are as non-invasive as possible, while achieving a high degree of accuracy. The needs for phenotyping tools dedicated to various and detailed traits to support the breeding of multi-purpose animals adapted to challenging and changing environments (associated with climate change, mitigation strategies, availabilities of feed resources) have not always been addressed because of a non-common research field and the lack of efficient connections between phenome and genome sciences. During the last decade, several attempts to align the needs for breeding and phenotyping tools based on a deep understanding of the relationships between biological traits have emerged, resulting in promising examples of traits especially developed to describe specific characteristics of animals. A first example is based on the partitioning of feed efficiency in growing pigs between what is due to the digestive utilization of diets and what is due to the metabolic utilization of nutrients. Based on the observation of a genetic contribution to the variability with which a pig digests a diet, a method based on a near infrared spectroscopy of a fecal sample has been developed to be able to breed pigs especially able to digest efficiently diets with a low nutritional value. A second example is the consideration of strategies to reduce methane emissions from dairy cattle in a breeding index together with economically important traits. The individual methane emissions of each cow have been evaluated thanks a method based on milk mid-infrared spectroscopy to document their genetic determinism. These two examples were supported by samples, measurements and data analyses relatively small in size in the way they only consider national or even regional populations of animals. These approaches can be further extended at the European level, also taking advantage of the differences in climate, species and breeds with their specific breeding strategies. Major advances in common trait definition and phenotyping procedures have pave the way to the development of a common infrastructure to integrate phenotyping, genomic and data research facilities. Such an infrastructure should be thought to efficiently develop in vivo and in vitro phenotyping, genomic tools, biobanking and data sharing and workflows so that it will be able to address complex research questions on farm animals and to translate results into impacts for animal breeding and farming.

Image-derived latent features as digital biomarkers for large-scale genetic evaluation

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Integrating high throughput phenotypic features into genetic evaluations is essential for improving prediction accuracy and capturing complex biological variation. Although image-derived features are widely used as proxies for ground-truth measurements, their potential as digital biomarkers in breeding programs remains largely unexplored. In this study, we leverage two deep learning architectures, Vision Transformer (ViT) and β-Variational Autoencoder (β-VAE), to extract interpretable latent features from porcine images and assess their genetic potential as digital biomarkers. The ViT models were pre-trained on the ImageNet dataset to generate high-dimensional image embeddings, whereas the β-VAE models were trained on our custom dataset using an encoder-decoder architecture that learns compact latent representations. Our approach processes segmented images to generate N-dimensional latent variables, with the number of features determined by the model architecture, ranging from 768 to 1024 for the ViT models and 128 for the β-VAE models. To evaluate how image-derived latent representations capture systematic effects, we computed attribute-specific correlation matrices for several key factors, including contemporary group, birth year and month, birth farm, gender, and litter. We then estimated the genetic parameters of each latent feature using linear mixed models to verify that image-derived features can capture a heritable genetic signal. Finally, we developed three methods to construct digital similarity matrices from these features, including correlation-based, cosine similarity, and RBF kernel formulations, and evaluated their consistency with traditional pedigree and genomic relationship matrices. The proposed approaches were evaluated using a large-scale dataset comprising 15.9M pedigree records, 561.7K two-dimensional side view body images from 24K individuals, and 17.7K genotyped animals. Our results demonstrated that image-derived embeddings capture high-resolution signals for environmental, temporal, and phenotypic information. Specifically, ViT-derived embeddings exhibited more distinct correlation structures for systematic effects than β-VAEs, even when using a pre-trained model. These latent variables exhibited moderate and heterogeneous heritability, with estimates ranging from 0.06 to 0.49, confirming their capacity to represent a significant genetic signal. Finally, similarity matrices constructed from ViT embeddings were more correlated with pedigree and genomic relationship matrices than β-VAE based approaches, with correlations ranging from 0.27 to 0.31. Overall, these results demonstrate that image-based latent features can function as digital biomarkers for genetic evaluations, offering a novel covariance component to augment traditional pedigree or genomic relationship matrices and enhance the partition of phenotypic variance.

Extending Single-Step Genomic Evaluations to Phenomics and Intermediate Omics: A Conceptual and Methodological Perspective

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Phenomic selection and the integration of intermediate omics information are emerging strategies to enhance the prediction of complex traits in animal breeding. Both approaches exploit high-dimensional biological data—such as infrared spectra, metabolomic or volatilomic profiles, transcriptomic measurements, and imaging-derived features—to capture biological variation not fully explained by traditional genotyping. These data layers contain functional and environmental information that often precedes or modulates genetic expression, providing complementary insights into the biological mechanisms underlying quantitative traits and opening new avenues for precision breeding. Despite their increasing empirical use, the conceptual and methodological link between such high-throughput data sources and conventional genomic evaluation frameworks remains underdeveloped. This work proposes a unified perspective that positions phenomic and omics data as relationship layers, analogous to genomic and pedigree-based relationships. This interpretation enables the joint estimation of random effects and the evaluation of individuals across multiple information sources within a single modeling framework. In conventional genomic evaluation, genotyped individuals contribute a relationship matrix reflecting additive genetic similarity, which can be extended in a single-step framework to include non-genotyped animals through pedigree information. By analogy, when phenomic or omics features are used to construct similarity matrices, the resulting kernels are often implicitly treated as if they arise from perfectly heritable signals. Recognizing, quantifying, and revisiting this assumption is essential for properly interpreting and extending single-step evaluations when integrating high-throughput biological data. Each data source – pedigree, genomic, phenomic, or -omic – defines its own relationship structure, which can be combined within multi-kernel mixed models or incorporated into neural-network architectures where intermediate features act as latent layers. Such multi-layer representations allow information to propagate across biological levels and between individuals, capturing both direct genetic effects and indirect effects mediated by functional or physiological traits. By formalizing phenomic and omics kernels as additional relationship layers, this conceptual extension provides a rigorous basis for combining heterogeneous data types, improving predictive accuracy, and enabling the inclusion of individuals with incomplete or missing measurements. The proposed concepts will be exemplified using Raman spectroscopical and volatilomic profile data associated with boar taint, illustrating how, potentially, high-throughput phenomic information can be operationalized within a single-step evaluation framework. This integration clarifies assumptions about heritability, information transfer, and biological relevance, supporting the development of flexible, data-rich, and biologically grounded multi-layer prediction systems for future livestock breeding programs.

Metabolic signatures after genetic selection of litter size homogeneity and resilience in a maternal rabbit line

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Selection for resilience in animal farming is an attractive strategy to ensure productivity under environmental challenges while promoting long-term welfare. Resilience is often defined as the ability to maintain production levels under environmental perturbations throughout the productive life, which is costly and difficult to measure. With the revolution in omics data, many studies have identified phenotypic biomarkers of resilience; however, their genetic basis and potential use for breeding are usually unexplored due to a lack of data or proper experimental design. A divergent selection experiment in rabbits at the University Miguel Hernández (Orihuela, Spain) for intra-dam phenotypic variance of litter size after correcting for year–season and lactation status produced lines with homogeneous and heterogeneous litter sizes. After 17 generations, the lines differed by 1.58 kits² (0.49 standard deviations), attributable to genetics. A preliminary study in generation 13 observed different inflammatory and immune responses between the lines targeting a few specific biomarkers (blood leukocyte counts, TNF-alpha, and C-reactive protein), although the full picture of the biological mechanisms underlying resilience remained poorly understood. This study compared the comprehensive plasma metabolic profiles of these two lines in two different generations (13 and 17) to identify plasma metabolites responding to selection for resilience. Candidate biomarkers were identified in generation 17 and subsequently validated in generation 13 to reduce false-negative detection rates associated with high-dimensional data ($p >> n$). Blood samples were collected 24 hours after first kindling from 40 randomly selected does per generation (13 and 17). Untargeted plasma metabolomics using a semipolar approach identified 1,397 metabolites in generation 13 and 1,170 in generation 17. Metabolite abundances in generation 17 were chi-squared normalised prior to a preliminary screening that retained 180 metabolites with relative abundance differential mean ($n = 69$) or variance ($n = 131$). Differential means were estimated with Bayesian linear models, while differential variance was assessed using Fisher's F-test. Lines were classified through logistic regression, partial least squares discriminant analysis (PLS-DA), and random forest following different transformations of the 180 metabolites: chi-squared normalization, pairwise log-ratio (LR), centered log-ratio, and additive log-ratio. Model performance was evaluated across 100 iterations of repeated 70:30 train–test splits, incorporating algorithm-specific feature selection. The highest classification accuracy was achieved by PLS-DA using LR. An additional PLS regression analysis identified informative metabolites related to resilience by overlapping pairwise LRs from PLS and PLS-DA ($n = 167$). This subset was then validated in generation 13 with common metabolites ($n = 16$). Consistent cross-generational differences were observed in LRs primarily composed of 3-hydroxy-4,5-dimethyl-3(5H)-furanone, but also creatine, 3-methylglutarylcarnitine, and N-(3-acetamidopropyl)pyrrolidin-2-one, linked to energy metabolism, immune regulation, and stress adaptation. The next step is to further investigate the biological link between these metabolites and resilience, and their potential in resilience-focused breeding.

Resolution matters: 16S rRNA and Shotgun Metagenomics in estimating microbiome-mediated genetic variance in Iberian pigs

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Recent developments in metagenomic technologies have facilitated the incorporation of microbial information into animal breeding strategies, providing new insights into the role of host-microbiome interactions in shaping phenotypic diversity. In this context, the present study evaluated and compared two sequencing methodologies, 16S rRNA gene sequencing and shotgun metagenome sequencing (WGS), to assess their capacity to characterize microbial and genomic contributions to meat quality and carcass traits in Iberian pigs. A total of 162 pigs were raised under standardized conditions at the Iberian Testing Center (Inga Food, Spain). Fecal samples collected before slaughter (≈365 days) were analyzed using nf-core/ampliseq (16S) and SqueezeMeta (WGS). After quality control, WGS retained 275 genera, 212 species, and 4527 KEGG Orthologs (KOs), whereas 16S retained 154 genera, 44 species, and 1775 KOs. Animals were genotyped with the Illumina GGP PorcineHD BeadChip (35,285 SNPs post-QC). Phenotypes included fatty acid (FA) composition of longissimus thoracis and carcass and primal-cut yields (ham, loin, shoulder). A three-step Bayesian framework (BGLR, R) was applied. First, microbiability was estimated using a mixed model with additive genetic and microbial random effects. Second, BayesC regression estimated microbe-specific effects including additive genetic effects and microbial abundances. Third, each microbe was modeled as a quantitative trait to estimate its additive genetic variance, allowing estimation of the fraction of trait genetic variance mediated through each microbe, and ranking taxa according to their contribution. Both approaches detected relevant microbial contributions. At the genus level, microbiability estimates were similar for FA traits (16S: 0.20–0.23; WGS: 0.22–0.24) and carcass traits (16S: 0.14–0.32; WGS: 0.14–0.20). At the species level, WGS yielded markedly higher estimates than 16S for FA traits (0.19–0.27 vs. 0.09–0.11) and carcass traits (0.14–0.20 vs. 0.07–0.11), except for ham yield (16S: 0.21; WGS: 0.19). At the functional level, estimates were comparable (16S: 0.16–0.33; WGS: 0.15–0.32), with 16S slightly higher for most traits. Microbe-specific regression coefficients showed strong heterogeneity, with few taxa explaining large proportions of microbiome-mediated genetic variance. At the genus level, Prevotella, Lactobacillus, and Streptococcus explained 37–93% depending on the trait. At the species level, Escherichia coli, Lactobacillus amylovorus, and Streptococcus gallolyticus accounted for 22–93% depending on phenotype. Overall, similar estimates at genus and functional levels indicate comparable performance of both methods at these resolutions. In contrast, substantially higher species-level estimates from WGS indicate stronger support for species-associated variance components, whereas lower 16S estimates reflect limited species resolution. Thus, WGS is required to detect species-level effects, while 16S is adequate for genus- and function-level analyses.

Does the inclusion of microbiome data enhance genetic evaluations? Evidence from GBLUP and GO-BLUP comparisons in beef cattle

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Genetic improvement in cattle relies on accurate prediction of estimated breeding values (EBVs) for traits of economic relevance, particularly those related to growth, feed efficiency, and carcass composition. Although genomic selection has substantially improved EBV accuracy over the past decades, the incorporation of additional layers of biological information may further enhance predictive performance. In this context, the rumen microbiome has gained increasing attention as a biologically meaningful intermediate phenotype that may partially mediate the relationship between host genotype and complex production traits. Therefore, integrating metagenomic information into genomic evaluation frameworks represents a promising strategy to refine selection decisions. The aim of this study was to evaluate whether the inclusion of rumen metagenomic data improves predictive accuracy compared to a conventional genomic best linear unbiased prediction (GBLUP) model, using a genomic-omic BLUP (GO-BLUP) approach for feed efficiency and carcass-related traits in commercial beef cattle. Data included 1,458 British Blue × Holstein crossbred animals genotyped for 65,782 SNPs, aged 213 ± 33 days at the start of the trial, all fed the same commercial diet and evaluated at a commercial test station. Feed intake and body weight were recorded during 4-week trial, allowing the estimation of feed conversion ratio (FCR), average daily gain (ADG), daily feed intake (DFI) and residual feed intake (RFI). At the end of the trial, intramuscular fat (IMF), eye muscle area (EMA), and subcutaneous fat thickness (SFT) at the 12th–13th rib were measured by ultrasound, rumen samples were collected via stomach tube for whole-metagenome sequencing, and carcass weight (CW) was recorded at slaughter. Predictive performance was assessed using a repeated external validation scheme in which 80% of the animals were randomly assigned to the training set and the remaining 20% constituted the validation set with masked phenotypes, replicated across 100 random partitions. All performance trait models included sex and batch as fixed effects, and diet composition variables and age as covariates. First, a GBLUP model with a genomic random effect was fitted. Subsequently, a two-step GO-BLUP approach was implemented. In the first step, a GBLUP model including genomic and microbial random effects was fitted to estimate direct genomic and microbiome variances using the GRM and MRM matrices. Estimated microbial values were then used as a phenotype in a second model including sequencing depth as a fixed effect and a genomic random effect to estimate microbiome-mediated genomic variance, thereby partitioning genetic and environmental components. Empirical predictive ability was calculated as the correlation between adjusted phenotypes and EBVs in the test set. Across traits, GO-BLUP yielded higher predictive ability than GBLUP in animals with masked phenotypes for ADG, with a mean improvement of +20% ($p < 0.001$), +8% for DMI ($p = 0.003$), and +10% for FCR ($p = 0.015$). MGs explaining the largest proportion of genetic variance for ADG and DMI were UBA2810 and Dialister. No relevant differences were observed for EMA, CW, SFT, IMF, DFI, or RFI. These results suggest that incorporating rumen metagenomic data improved prediction accuracy for efficiency traits, supporting microbiome-informed genomic evaluation through GO-BLUP.

Evaluating the Contribution of Microbiome Information to Genomic Prediction for Growth, Feed Intake, and Carcass Traits in Angus Cattle

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Incorporating ruminal and gut microbiome information into genomic models enables partitioning of genetic variation into host and microbiome contributions, potentially enhancing the accuracy of genetic evaluations. We aimed to evaluate the impact of including ruminal and fecal microbiome similarity matrices in BLUP-based models for predicting genomic breeding values (GEBV) in American Angus cattle. Fecal and ruminal samples were collected from 1,460 yearling Angus bulls across eight U.S. feed efficiency centers. After data editing, samples from animals not registered with the American Angus Association and those that did not meet quality standards for microbiome analysis were removed. A total of 906 (ruminal) and 913 (fecal) animals, born between November 2020 and May 2022, remained for further analyses. Amplicon sequence variant (ASV) abundances were inferred using the DADA2 and Greengenes2. After rarefying samples to 10,000 reads and filtering ASVs with total abundance <1,200 and prevalence <5%, 1,451 ruminal and 1,388 fecal ASVs were kept for the analysis. Genomic prediction was performed for four economically important traits: weaning weight (WW), post-weaning gain (PWG), dry matter intake (DMI), and ultrasound backfat thickness (uFAT). Predictive performance of GEBVs was evaluated using the linear regression (LR) method implemented in validationf90 in single-trait models, including fixed effects of contemporary group, age classes (for DMI), overall mean, and birth year. The focal group was comprised of animals born in April and May 2022, totaling 85 animals for the ruminal group and 86 for the fecal group. Four models were considered: GBLUP, additive genetic effects only (u); GMBLUP, additive genetic effects not mediated by the microbiome (ur); mBLUP, which uses the predicted microbiome effects from the GMBLUP model as pseudo-phenotypes to estimate additive genetic effects mediated by the microbiome (um), and COMB, combining microbiome-mediated and non-mediated components (ut = ur + um). In both ruminal and fecal datasets, GBLUP and GMBLUP showed similar predictive performance, indicating that the host genetic component not mediated by the microbiome accounts for most of the stable signal captured in genomic prediction. The BLUP model showed reduced accuracy and greater dispersion, particularly for PWG and uFAT, suggesting that microbiome-mediated effects have limited predictive power when considered alone. However, combining microbiome-mediated and non-mediated effects improved prediction stability. The COMB model consistently achieved accuracies comparable to or slightly higher than those of GBLUP, with low bias and dispersion across most traits. Improvements were more evident for WW and PWG, whereas DMI and uFAT showed limited gains from microbiome inclusion. Overall, the results indicate that microbiome information alone does not improve genomic prediction, but when combined with host genomic effects, it provides greater stability, supporting the microbiome as an additional biological factor influencing economically important traits in Angus cattle.

Integrating functional SNVs with mRNA isoform expression in A1A1 and A2A2 Holstein dairy cows

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The A2 β-casein variant has gained considerable interest due to proposed health benefits, contributing to the increasing frequency of the A2A2 genotype in dairy herds. Although the A1/A2 substitution in the β-casein gene (CSN2) does not directly modify gene regulation, previous studies have reported differences in gene expression, especially at the mRNA isoform level, between A1A1 and A2A2 cows. Thus, this study aimed to investigate whether Holstein cows with A1A1 and A2A2 β-casein genotypes exhibit distinct single nucleotide variants (SNVs) using RNA-Seq data derived from milk fat globule (MFG). Variant calling was performed using CLC Genomics Workbench, and were classified according to their predicted effects: (i) amino-acid change (AAC), (ii) splice site effect (SSE), and (iii) variants exhibiting both effects. In A1A1 cows, 3,033 SNVs were predicted to generate AAC, 170 SNVs were predicted to cause SSE, while 59 SNVs showed both effects. In A2A2 cows, 3,256 SNVs were predicted to generate AAC, 202 SNVs to cause SSE, and 75 SNVs to exhibit both effects. The Ensembl Variant Effect Predictor (VEP) was used to functionally annotate all variants according to their predicted consequence types. Regarding SNVs leading to AAC, only those with SIFT (Sorting Intolerant From Tolerant) < 0.05 were retained, as these values indicate a high probability of affecting protein function. SNVs leading to SSE were integrated with previously analysed differentially expressed (DE) mRNA isoforms, and only those affecting genes with at least one DE isoform were further analysed. Functionally relevant genes affected in A1A1 group of cows were associated with mitochondrial oxidative phosphorylation (OXPHOS) (NDUF subunits, COX7A2 and ATP5PF), together with genes related to mitochondrial integrity and function (SLC25A44, PTCD1, PISD and IMMT) and lipid metabolism (ACSL1, MFGE8). A2A2 genotype-associated variants were also identified in genes related to mitochondrial function (OPA1, PTCD1, SLC25A46, NSUN3 and SIRT4), lipid metabolism (CPT1B, LPIN1, FASN, SPTLC2), as well as vesicle trafficking and secretory pathways (MVB12B, LRRK2, ABCA2, ABCA5, SEC31A and ABCG2). A deeper functional interpretation of these genotype-associated SNVs in expressed regions of the genome will clarify their biological relevance and the pathways potentially affected in the context of selective breeding towards the A2A2 genotype in Holstein dairy cows.

Effectiveness of DNA Sequencing and Biochemical Methods in Characterizing Bovine Milk Protein Variants

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Bovine milk proteins are highly polymorphic, and several variants are known to influence milk processing properties and nutritional quality. Milk protein variants can be identified either through DNA sequencing or biochemical analysis, but concordance between these approaches has not been extensively studied. This study aimed to evaluate the effectiveness of DNA-based predictions and biochemical analyses of milk samples for the identification of milk protein variants. To achieve this, we compared predictions based on whole-genome sequencing data with average coverage of 10× with RP-HPLC analysis of milk samples from 178 cattle originating from different European and African breeds. LC-MS was subsequently used for a subset of the milk samples to confirm new DNA-predicted variants and resolve additional isoforms detected by RP-HPLC. Our results show that DNA sequencing shows an high accordance of 92%-97.2% compared with RP-HPLC for the major known protein variants. However, SNP read depth had a substantial impact on the discordance and on the call rate. In our data set a depth of four reads represented a minimum requirement for reliable protein variants genotyping. Moreover, 6 out of 10 DNA-predicted rare variants were confirmed using LC-MS, while the remaining 4 variants could not be validated successfully due to insufficient read depth at corresponding SNPs or due to sequencing errors. Finally, protein analysis also identified variants (αS1-CN A and β-CN A3) that were not detected by DNA sequencing due to the difficulties in identifying newly splice variants and low-quality SNP calling, respectively. This study provides a systematic evaluation of the concordance between DNA-based inference and biochemically protein-level identification of milk protein variants. We demonstrate that moderate DNA sequencing coverage (~10×) achieves comparable agreement of more than 92% compared with RP-HPLC. However, accurate identification of protein variants from DNA sequence data remains challenging, especially for low quality SNP calling and incomplete functional annotation of splice variants. Increasing the stringency of the read depth can reduce the discordance but also decreases the call rate. Keywords: Protein variants, DNA sequencing, RP-HPLC, LC-MS

Multi-evidence driven triage of candidate genes for feather pecking behaviour in chickens

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Feather pecking in laying hens is a complex, multifactorial behavioural trait with a genetic component that significantly impairs animal welfare and increases energy requirements, resulting in lower resource efficiency. Since divergent selection for high and low feather pecking behaviour led to the establishment of two distinct lines with very high and no propensity for the behaviour, respectively, a causative connection between genetics and the behaviour is evident. Hence, we hypothesise that functional genetic variants that alter the structure, stability, or expression of proteins in key neurobiological pathways are likely to influence individual variation in feather-pecking behaviour in laying hens. Our objective is to prioritise a short list of candidate haplotypes for experimental validation, recognising that many protein-altering variants may be phenotypically silent. For this, whole-genome sequences from hens of high and low feather-pecking lines (>15 generations of selection; n=49) were contrasted using population differentiation to identify strongly diverged loci. Haplotype-aware protein sequences were reconstructed, and candidates were ranked using an integrated evidence score. Previously called SNPs, small indels (<50 bp), structural variants and tandem repeats were analysed by computing Fixation indices (FST) with PLINK. Haplotype-aware protein prediction was performed with Haplosaurus (Ensembl VEP). Variant consequences were annotated with VEP/snpEff; missense impact with SIFT 4G and PROVEAN; splice effects with SpliceAI; disordered regions with IUPred3 and motif mapping with ELM. Protein structures/complexes were modelled with AlphaFold3 and stability/interface effects estimated with FoldX; interaction partners were obtained from STRING; tissue expression context from Bgee and Expression Atlas. Evidence signals were standardised and combined as a weighted score. Differentiation was quantified by per-variant FST; highly differentiated variants were defined as FST>0.9 and summarised descriptively. Across 49 sequenced hens, 125,000 highly differentiated variants (FST>0.9) were detected between lines, including 681 protein sequence–altering haplotypes (up to 14 missense variants per haplotype as well as indels). SpliceAI predicted 66 variants with potential splice disruption. Preliminary joint analysis of all datasets led to the production of a prioritised list of candidate genes for feather pecking behaviour, with the top candidate gene encoding the Apolipoprotein B (APOB). The APOB haplotype harbours 14 missense mutations, which led to a significant score in four of the above mentioned analyses. Besides being the highest-scoring gene in this study, APOB has been linked to aggressive behaviour in humans by two independent studies. This makes APOB a top candidate for experimental validation of its causality in feather pecking behaviour. This multi-evidence, tool-based triage integrates sequence, structure, regulatory and biological-context evidence to prioritise a manageable set of neurobiologically plausible candidates for functional testing, explicitly addressing that structural changes alone may not translate into behavioural phenotypes. The approach is species independent and is applicable to a wide range of behavioural disorders and can be easily adapted to any complex genetic disorder with a measurable phenotype.

Utilising genotype and transcriptome information for feather pecking analysis in laying hens

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Feather pecking in laying hens represents a significant welfare concern and leads to substantial economic losses. Although genome-wide association studies have identified genomic regions linked to this behaviour, pinpointing the underlying causal genes remains difficult. Transcriptome-wide association studies (TWAS) address this challenge by combining genotype data with gene expression profiles to evaluate whether the genetically regulated component of gene expression is associated with the trait. We applied an individual-level TWAS framework to feather pecking to identify candidate genes and biological pathways that may mediate genetic risk, while also evaluating multiple TWAS modelling approaches to ensure robust and reliable inference. We will additionally integrate genotype and transcriptome data into mixed linear models to partition phenotypic variance into mentioned components and assess predictive performance through cross-validation based on genomic and transcriptomic similarity. Data were derived from a half-sib (HS) population from the 15th generation of a long-term feather pecking selection experiment of Lohmann Selected Leghorns. Individual-level genotype and RNA-seq data from 48 birds were used to train cis-genetic prediction models of gene expression (variants within a defined window around each gene) following genotype quality control and expression normalization. The genetically regulated component of expression was then imputed into an independent set of 441 genotyped birds from the same population and evaluated for association with feather-pecking phenotypes. Multiple testing was addressed using Bonferroni correction. In this analysis, thousands of genes yielded usable expression prediction models and were subsequently evaluated in the association cohort (2,990 genes with BLUP; 8,421 with nonparametric Bayesian DPR; and 9,884 with elastic net). The number of Bonferroni-significant genes differed across models (472, 2,172, and 1,971 respectively), indicating that TWAS findings may be sensitive to modelling choices when the training sample size is small (n=48). These preliminary findings support the utility of TWAS for prioritizing candidate genes, while underscoring the need for further enhancement. We will enhance adjustment for covariates, population structure, and relatedness to ensure reliable results. Genes supported across analyses will be prioritized and interpreted through enrichment and functional context. The expected outcome is a reproducible workflow and candidate genes for feather pecking in chickens and supporting welfare-oriented breeding and management decisions.

From genotype to phenotype: mapping shared regulatory architecture of cattle metabolome and transcriptome

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In genomic studies identifying the pleiotropic genetic regulators helps to elucidate the molecular mechanism underlying complex phenotype and facilitate the identification of functional causal variation. These kinds of genetic regulators can be identified by colocalizing the regions from multiple genomic studies. Colocalization is a robust statistical approach used to determine whether a shared causal variant underlies associations across multiple omics layers, effectively distinguishing pleiotropy from coincidental linkage. In this study, we performed colocalization analysis of metabolite quantitative trait loci (mQTL) derived from 147 plasma metabolites in 325 individuals of Charolais × German Holstein crossbred population and expression quantitative trait loci (eQTL) from RNA-seq of liver tissue in 357 samples, across diverse cattle breed populations. For both datasets the genotype data consists of whole genome sequence imputed genotypes. The colocalization analysis was carried using the coloc.abf() function from R package coloc. This function assumes a single causal variant for each trait and calculates the posterior probabilities of different configurations using a Bayesian approach. For each selected region across both datasets, the results summary generates a vector of the common SNPs tested per region and five posterior probabilities for causal association ranging from PP.H0 to PP.H4, where PP.H0 represents the probability of having no causal variant and PP.H4 the probability of having a causal variant. For colocalization we considered only the significant loci ($P<5\times10^{-8}$) associated to different plasma metabolites from mQTL dataset. Similarly, the eQTL dataset comprises summary statistics of significant variants (adjusted $P<0.005$) derived from gene-level cis-eQTL conditional analysis with multiple independent signals per gene which were based on conditional ranking. To perform the colocalization, coloc.abf() was provided with significant SNPs and a 250 kb buffer on either side, ensuring the inclusion of the full genomic context for both mQTL and eQTL signals. We cross-referenced the allele assignments for each variant between the two datasets to confirm concordance and maintain data integrity. We identified 42 significant colocalized regions (PP.H4>0.8). Most of these are located on BTA-11 between 3.2–3.8 Mb and BTA-15 between 72–82 Mb. In previous studies a significant QTL was identified at BTA-11 3.6 Mb which was associated to CNAG3. The CNGA3 has been identified as a key marker for feed efficiency in beef cattle, likely influencing Residual Feed Intake (RFI) through the enrichment of multiple ion transport and signal transduction pathways. The BTA-15:72–82 Mb region is identified as a QTL hotspot in CattleQTLdb, containing eight loci associated with average daily gain, along with two loci related to traits, serotonin levels and antral follicle count. Future research will investigate this region further, to uncover potential regulatory pathways and networks influencing the metabolites and genes expressed in these cis-eQTL regions.

Comparative profiling of the bovine plasma proteome using Olink proximity extension assay and LC–MS/MS before and after calving

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The transition period around parturition in dairy cattle involves major metabolic, endocrine, and immune adaptations reflected in the plasma proteome. However, the extent to which different proteomic platforms capture these changes in bovine plasma remains unclear. The objective of this study was to compare a human-targeted proximity extension assay (Olink Explore 3072) with discovery-based liquid chromatography–mass spectrometry (LC–MS/MS) for plasma protein detection and differential expression analysis in dairy cows before and after calving. Plasma samples from eight cows before and after calving were analysed using the Olink Explore platform (targeting 2,932 human proteins) and LC–MS/MS. Olink data were quality-controlled and filtered to retain proteins detected in ≥50% of samples. LC–MS/MS data were generated using a data-dependent acquisition (DDA) workflow and processed with PEAKS X Pro for label-free quantification, resulting in 300 quantified proteins ≥ 2 unique peptides. To ensure statistical consistency, differential protein abundance for both platforms was evaluated using linear models implemented in the limma package in R, including cow identity as a blocking factor to account for paired samples collected before and after calving. Using Olink, 925 proteins were detected, of which 22 showed significant differential abundance (FDR < 0.05) with 12 proteins increased after calving (e.g., FGF21, NOS2 and CHGA) and 10 decreased (e.g., OXT, DKK1 and COL9A1). Cross-species ortholog mapping identified 865 bovine orthologs among the detected proteins, with 795 showing ≥70% sequence identity between human and bovine proteins, indicating strong conservation. In comparison, LC–MS/MS identified six significant differential abundance (FDR < 0.05), including APOM, GC, CPN1, AGT and APOF, which were more abundant after calving, while VNN1 showed higher abundance before calving. Notably, GC (vitamin D–binding protein) has shown to be associated with mastitis susceptibility in dairy cattle. Only fourteen proteins were quantified by both technologies. Comparison of fold changes revealed moderate agreement (60%) in the direction of change between platforms. Differences between platforms reflect their distinct analytical principles. Olink uses antibody-based targeted detection and captures low-abundance regulatory proteins, whereas LC–MS/MS provides unbiased identification of endogenous bovine proteins but preferentially detects higher-abundance plasma proteins; species-specific differences between human assay targets and bovine proteins may influence Olink detection. Together, the results demonstrate that Olink and LC–MS/MS provide complementary insights, improving the characterization of the bovine plasma proteome during critical physiological transitions. Integrating targeted and discovery proteomics approaches, therefore offers a powerful strategy for improving characterization of physiological changes around calving and identifying candidate biomarkers relevant to dairy cattle health.

Genetic Score Omics Regression to Map Genes Underlying Stillbirth and Gestation Length

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Since the introduction of SNP-chip genotyping, the understanding of health and yield traits in farm animals has markedly improved. Transcriptomics represents the next layer of the omics cascade, providing direct measures of gene activity. Recent efforts in animal science have focused on aggregating public RNA-seq data across tissues to create larger, more powerful datasets. A notable example is CattleGTEx, a multibreed collection of RNA-seq data and imputed genotypes in cattle. To link gene expression data and quantitative traits, the Transcriptome-Wide Association Study (TWAS) is a common method. It trains a prediction model of genetically regulated expression in a usually small group of animals with measured gene expression and predicts gene expression levels into a larger group of phenotyped animals, where associations between predicted expression and phenotypes are tested. The recently introduced Genetic Score Omics Regression (GSOR) method is a reversed approach and uses SNP-effects trained on a much larger sample size for phenotype prediction and predicted local genomic breeding values (EBVs) into samples with assayed gene expression. Then, GSOR estimates the correlation between measured gene expression and estimated phenotypes based on gene-specific local EBVs. We applied GSOR to the direct and maternal effects on stillbirth and gestation length in German Holsteins. The expression sample sizes in CattleGTEx for the tissues of blood, liver and uterus varied between 359 and 945. The number of cows with de-regressed proofs ranged from 36,502 to 192,880. Based on these and a set of imputed and LD-pruned WGS genotypes, BayesR3 was used for effect estimation of approximately 2.5 million SNPs effects for stillbirth and gestation length traits. Local EBVs were then computed based on the effects of SNPs located in the gene and within ±1 Mb. Correlations between these local EBVs, and the CattleGTEx gene expression with an FDR-corrected p-value < 0.05 were deemed significant. Results were further filtered for a local EBV variance above the 75th percentile, retaining only genes with meaningful variation. Summary-based Mendelian Randomization (SMR) was used as a third filtering step to prioritize genes with evidence for causality. The remaining trait-associated, potentially explanatory, genes were interpreted based on PubMed (NCBI). Most identified genes were associated with gestation length and only a few with stillbirth. This was expected based on the lower heritability of stillbirth compared to gestation length. The biological relevance of the associations found by GSOR is, for example, supported by correlated genes in the liver, that have been associated with fat metabolism by previous studies. Another correlation was identified between a gene's expression in blood and the maternal effect on gestation length. Here, the literature suggests an important role in placental nutrient transport with effects on birth weight. In summary, we successfully applied GSOR for stillbirth and gestation length, identifying genes and underlying metabolic pathways affecting these traits.

Transcriptomic signatures associated with intramuscular fat deposition in the Black Slavonian pig

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Intramuscular fat (IMF) is a key determinant of pig quality, strongly influencing sensory attributes such as flavor, tenderness, and juiciness. Although the molecular mechanisms regulating IMF deposition have been extensively studied in modern commercial pig populations, considerably less information is available for traditional and locally adapted breeds. Native pig populations often possess distinct metabolic and physiological characteristics, making them valuable models for investigating alternative regulatory pathways involved in lipid accumulation in skeletal muscle. The present study explored transcriptomic differences associated with divergent IMF levels in the Black Slavonian pig, an indigenous Croatian breed recognized for its high-quality meat products. Muscle tissue samples from the Longissimus thoracis et lumborum were obtained from fourteen animals displaying contrasting IMF content. The IMF percentage was quantified from the same muscle region 24 hours post mortem using the Soxhlet extraction procedure. RNA sequencing was applied to profile gene expression patterns between the two phenotypic groups. Differential expression analysis revealed over 500 genes showing significant expression differences ($p \leq 0.05$), of which 457 remained significant after adjustment for multiple testing. Animals with higher IMF content showed increased expression of genes involved in lipid metabolism and adipocyte differentiation, including SCD, ADIPOQ, CIDEC, and FABP4, as well as activation of regulatory pathways linked to PPAR signaling and fatty acid synthesis. Conversely, several genes associated with muscle fiber organization and oxidative metabolism were relatively suppressed in the high-IMF group. Functional enrichment analyses indicated that IMF accumulation in skeletal muscle is associated with coordinated regulation of multiple biological processes. In addition to lipid metabolic pathways, significant enrichment was observed for pathways related to extracellular matrix remodeling, vascular development, and cell–cell signaling. Several extracellular matrix components (e.g., LAMA1, TIMP4) and angiogenesis-related genes (FGF2, NRP1) displayed elevated expression levels, suggesting that the expansion of intramuscular adipocytes is accompanied by structural remodeling of muscle tissue and enhanced vascularization. Also, some of the highly ranked differentially expressed genes have not previously been widely implicated in IMF deposition in pigs. Among them, EHD, NRG4, UTRN, FLNA, and HMCN1 may represent novel candidates potentially involved in processes such as membrane trafficking, paracrine communication between adipocytes and muscle fibers, cytoskeletal organization, and extracellular matrix dynamics. These findings suggest that IMF regulation in local pig breeds may involve partially distinct molecular mechanisms compared with those described in intensively selected commercial lines. The results expand current knowledge on the molecular architecture of meat quality traits in indigenous breeds and highlight potential candidate genes and pathways that could be considered in future breeding programs aimed at improving pork quality while preserving valuable genetic diversity.

Genetic and genomic strategies for advancing sustainable beef-on-dairy systems

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Although beef-on-dairy is not a new practice, its adoption has increased recently with the widespread availability of sexed semen. In this system, the best dairy females are used to generate replacement heifers, whereas beef semen is used on the remainder to enhance the value of surplus calves. This practice has strengthened the connection between the dairy and beef sectors; however, the initial choice of beef semen was not driven by genetics. Incorporating genetic and genomic strategies into beef-on-dairy programs is essential to ensure the long-term sustainability of the system. As breeding companies and breed associations began designing breeding programs or selection indices specific to beef-on-dairy, the performance of crossbred products improved to the point that consumers have a similar experience to that of traditional beef products. While carcass quality is a primary concern for the beef sector, dairy producers are particularly concerned about fertility, calving performance, and calf health. Fertility concerns may have arisen because beef sires were frequently used in cows already identified as problem breeders. In a study on conception rate (CR) in the USA, we observed mean CR of 33.77% and 34.29% for breedings with Angus and a Holstein sires mated to HO cows, respectively. For matings with Holstein heifers, the CR were 52.96% and 55.34%, in the same order. The average number of services was 42% and 47% higher for Holstein cows and heifers mated to Angus bulls, respectively. Because crossbreeding can influence gestation length as well as calf weight and size, potentially affecting the productive life of dairy cows, calving ease (CE) has become an important trait in beef-on-dairy systems. When investigating the genetic background of CE in beef-on-dairy crosses, we observed that, on average, the incidence of unassisted calvings in the first (first three) lactation was 83% (88%) for Holstein and 86% (90%) for Jersey cows bred to Angus, Charolais, and Simmental beef bulls. The highest proportion of assisted calvings occurred in Holstein heifers bred to Charolais bulls (27%) and Jersey heifers bred to Simmental bulls (22%). Overall, Holsteins showed a higher incidence of assisted calvings. Among the top 5% and bottom 5% of bulls based on genetic evaluation, the proportion of progeny with easy calving was 91% and 81%, respectively, demonstrating the value of estimated breeding values for identifying superior bulls. However, as the heritability of CE is low, at around 0.014, genetically improving CE would require large amounts of data and genomic information. Some of the issues contributing to the low heritability and, therefore, slower genetic progress include: the lack of data sharing between the dairy and beef sectors, which results in a complete pedigree on one side but a shallow pedigree on the other; the missing pedigree structure, which prevents information from flowing among animals; the complete disconnection between the beef and dairy pedigrees; and difficulty in sharing genomic information between the sectors. In this talk, we will discuss genetic and genomic strategies to optimize beef-on-dairy systems while balancing the interests of both the dairy and beef sectors. Particular attention will be given to opportunities for improving genetic evaluations through large-scale phenotyping, stronger pedigree integration, genomic selection, and cross-industry data sharing.

Balancing profit and calving performance: New breeding values for Beef-on-Dairy sires

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The use of Beef-on-Dairy (BoD) matings has expanded markedly in recent years. In Germany, BoD accounted for approximately 25% of all inseminations in 2025. Economic incentives include comparatively low semen costs and higher market prices for crossbred calves. In combination with female-sexed dairy semen and herd genotyping, BoD enables targeted mating strategies that enhance genetic gain in dairy herds while generating additional revenue. Until recently, beef sire selection for BoD matings was based mainly on phenotypic information. In 2025, Germany implemented new conventional breeding values for beef sires in dairy herds to support more informed and precise selection. New traits comprise calf weight and calf revenue, complemented by the direct calving traits calving ease and stillbirth. Data foundation for the breeding values of calf weight and calf revenue consisted of auction records from 116,419 BoD calves born between 2016 and 2025 with recorded sale weight and realized revenue at 14 to 90 days of age. Only calves sold on farms with complete records were included. Variance components were estimated using a bivariate multi-breed animal model with a five-generation pedigree. Fixed effects included age class × birth year × sex, herd × birth year and sire breed. Multi-breed, multi-trait breeding value estimation was subsequently performed, and the sire breed effect was incorporated into the final breeding value of each sire. The economic-related breeding values are reported on a natural scale: kilograms (kg) for calf weight and euros (€) for calf revenue. Calving-related breeding values are currently based on the genetic evaluation for Holstein cattle and are expressed on a relative breeding value scale. All BoD breeding values are calculated on a specific reference basis comprising BoD calves born within the last five years, ensuring comparability within and across breeds. The basis includes the main BoD breeds: Belgian Blue, Simmental cattle (beef type), Angus, Limousin, Charolais, Uckermärker, Blonde d'Aquitaine and INRA 95. Published breeding values reveal clear breed differences. Across breeds, mean breeding values relative to the BoD base population were 0.2 kg for calf weight and −32.6 € for revenue. Within-breed means ranged from −7.4 kg (Angus) to 4.2 kg (Blonde d'Aquitaine) for weight and from −154.1 € (Angus) to 39.1 € (Belgian Blue) for revenue. Reliability was high, averaging 89% for weight and 85% for revenue. Correlations between breeding values for calf weight and calving difficulty ranged from −0.17 to −0.70, indicating antagonistic relationships between growth potential and calving ease. For calf revenue, correlations with calving ease ranged from −0.33 to 0.23. These results confirm breed-specific differences and highlight the trade-off between economic and functional traits. The newly implemented BoD breeding values provide a robust tool for optimized beef sire selection in dairy herds, promoting economically valuable calves while safeguarding calving performance.

Early lactation prediction of extended lactation milk yield class using random forest models in pasture-based dairy systems

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In dairy cows, extended lactations (EL; milking for a period longer than the standard seasonal pattern) are a potential option for reducing the number of calvings in a cow's lifetime, and therefore, the number of surplus, non-replacement calves produced annually. However, the suitability of individual cows for an EL system is variable as their total milk production must align with the associated operational costs. The classification of high and low yielding animals could inform suitability of individual cows or a herd for maintaining a profitable farm business under EL. Early prediction of total EL milk yield is especially important within a seasonal pasture-based system, where breeding occurs within a seasonally concentrated timeframe and decisions to delay breeding, are made during early- to mid-lactation. Decisions for EL systems are made largely on a herd-level basis, with limited test-day data available before determining intended mating, and therefore EL cows. Earlier work indicates that there is potential to use early lactation data to predict total milk yield, however, to our knowledge, no studies in pasture-based cows have further attempted to build these models. Machine learning methods such as random forest techniques could be used to develop predictive models for cow performance. The aim of this study was to use early lactation curve features to predict cows as high- or low-yielding when managed for extended lactation. Milk yield records were obtained from dairy cows in New Zealand from a range of ages (2 to 10 years) and predominantly Holstein, Jersey and Holstein-Jersey crossbred breeds that had completed a 415-to-720-day lactation. In total, 5,226 EL records with at least seven herd tests were modelled with fifth order Legendre polynomials and daily yields estimated from individually modelled lactation curves. High and low yielding classes of average daily milk yield were determined based on a range of top and bottom percentiles of average daily milk yield for the length of each EL, then the EL records was split into training (70%) and test (30%) datasets. Several random forest models were then trained to predict EL yield class using animal characteristics, average daily herd production and modelled lactation curve features from the first 100 days. Variable importance measures were used to assess which aspects of the early lactation curve shapes were most strongly associated with EL yield performance. The performance of each model was evaluated using the test dataset and a confusion matrix and the best performing model selected. The models provided strong classification performance (balanced accuracy ~ 90%) indicating that early lactation features and cow-level characteristics such as breed and age, could provide insight into EL performance. Total yield to 50 and 100 days were also important features, suggesting that higher yielding cows early in lactation were likely to have a greater than average daily yield that can be sustained throughout the remainder of the lactation. Another predominant feature of importance was the average daily yield of the herd to 100 days, indicating that some herds were more likely to have suitable cows for EL than others. These results are consistent with prior research and support the potential use of machine learning methods to assist early decision making for EL selection in pasture-based systems. Prediction of suitable EL animals would help make informed decisions regarding breeding management and strategic use of EL at an individual cow and herd level.

Comparative Machine Learning for predicting late-lactation productivity in dairy cows.

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Extended lactations offer opportunities to increase cow lifespan by reducing the frequency of risk events related to calving, improve farm efficiency by reducing the labour associated with calving, and improve total milk produced per unit of time by reducing the relative number of dry-days. However, an extended lactation also has potential drawbacks: some cows may exhibit low milk production in late lactation or accumulate excessive body condition, which has been associated with impaired metabolic status after subsequent calving. Moreover, since pregnancy lasts around 9 months, insemination decisions must be made well in advance, under substantial uncertainty about future milk production. Conventionally farmers start inseminating their cows from day ~60 in lactation onwards. Successful implementation of an extended lactation management therefore requires timely selection of suitable cows and decision support when to inseminate them. Thus, using the cow information available before 60 days in lactation to predict lactation outcomes to select the suitable cows for an extended lactation could be valuable to farmers. Predicting future lactation performance from early lactation milk production was formulated as a time-series classification task. Daily milk yield during the first 60 days in milk (DIM) was used as input to predict late lactation production, defined as the cumulative milk yield between 250 and 257 DIM. This period was used as a proxy for lactation outcome while minimizing potential gestation effects. Lactations where classified using a predefined production threshold, separating cows for which extended lactation is advisable from those for which conventional insemination strategies are preferred. In this study, seven established time-series classification methods were evaluated on data consisting of a period of ~4 years from Dutch commercial dairy farms including 6226 lactations of at least 264 days which is the minimum length to be considered in this experiment. The following models were selected: Time-series forest classifier.Bag of Patterns Classifier.K-Nearest Neighbour time series classifier.Learning Shapelet Classifier.Ensemble of Bag of Symbolic Fourier Approximation Symbols. The Elastic Ensemble of time series distance measures.Hierarchical Vote Collective of Transformation-based Ensembles V2.These models represent distinct time-series classification paradigms: whole sequence-based, interval-based, shapelet-based, dictionary-based and hybrid approaches, enabling a structured comparison of how different temporal representations contribute to predictive performance. Model performance was evaluated using 5 fold grouped cross-validation with folds defined at farm level to prevent data leakage. Across all evaluated thresholds, the Time-series forest classifier, the interval-based paradigm, consistently achieved the highest predictive performance (accuracy: 0.852 ± 0.043, f1-macro: 0.607 ± 0.046, brier loss: 0.111 ± 0.026, AUC: 0.778). In conclusion, the interval-based representations of early lactation milk yield captured the most informative temporal characteristics. Future work integrating biologically relevant features, like autocorrelation or the log variance of milk yield, may further enhance predictive performance and support the development of decision support tools.

Impact of the use of Beef-on-Dairy on milk yield, calving traits and culling risk of the cow in subsequent lactation

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The use of Beef-on-Dairy (BoD) has expanded markedly in recent years, driven by the enhanced economic value of crossbred calves and the widespread adoption of genotyping in dairy herds. Although the monetary advantage associated with BoD calves is widely recognized, little is known about the long-term consequences for the dam. This study aimed to evaluate the effects of different beef sire breeds on subsequent milk performance, calving traits, and functional longevity of Holstein Friesian cows that gave birth to BoD calves, in comparison with cows that had purebred calves. A total of 2,189,503 calving events from across Germany, recorded between 2015 and 2024, were analysed. Of these, 1,358,212 were in second parity and 831,291 in third parity. Beef sire breeds considered were Angus, Blonde d'Aquitaine, Charolais, INRA95, Limousin, Uckermärker and Belgian Blue. Traits analysed included milk, fat and protein yield in the subsequent lactation, as well as dystocia, stillbirth, and culling probability. Production traits were evaluated using mixed linear models, while calving traits and culling probability were analysed by logistic regression. The models accounted for fixed and random effects such as herd, calving year and season as well as the genetic potential of the dam. In second lactation, cows inseminated with beef sires exhibited significantly lower milk yield compared with cows that produced purebred calves, with the greatest reductions observed for INRA95 (-638 kg), Angus (-433 kg) and Belgian Blue (-222 kg). Similar patterns were found for fat and protein yield. In third lactation, the magnitude of these effects was even more pronounced. Dystocia had a substantial negative impact on milk yield (reduction of -218 kg in second and -205 kg in third lactation). Culling risk was increased for most beef breeds, particularly Charolais, Blonde d'Aquitaine, Uckermärker and Belgian Blue, with higher probabilities occurring in early and late stages of lactation. Cows that gave birth to BoD calves exhibited significantly higher odds of dystocia compared with cows that produced purebred Holstein-Friesian calves. The highest odds ratios were observed for Charolais (OR = 6.79), followed by Belgian Blue (OR = 4.54) and Uckermärker (OR = 3.91). The risk of stillbirth was significantly higher for cows that gave birth to Blonde d'Aquitaine (OR = 1.80), Charolais (OR = 1.64), Belgian Blue (OR = 1.52) and Uckermärker (OR = 1.39) calves. In contrast, cows that produced Angus, INRA95 or Limousin calves did not differ significantly in stillbirth probability from those giving birth to purebred Holstein calves. In conclusion, although the breeding strategy BoD enhances the economic value of calves, cows that give birth to crossbred calves of certain breeds are at greater risk for calving difficulties, exhibit an increased likelihood of culling, and show reduced milk production in subsequent lactations. These results highlight the importance of thoughtful and carefully planned sire selection to balance economic benefits with animal welfare, longevity and production efficiency in dairy systems.

Splitting the bill at the gate: Pricing system to reward management and genetic potential of Beef on Dairy calves

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Calves enter the beef chain with very different starting conditions, reflecting variation in colostrum management, early nutrition, health status, genetics, and age at sale. These early-life factors are well known to influence health, mortality, growth rate, feed efficiency, slaughter weight, and carcass value. The Danish market has traditionally relied primarily on live weight, breed and sex premiums, providing limited incentive to optimize individual calf quality. This contribution discusses the rationale and implications of implementing a value-based pricing model on the Danish calf market. The tool, currently available for all Danish farmers via the Danish Dairy Management Software (DMS), predicts expected slaughter weight at 300 days using individual age and weight at transfer. It aims to reflect the calf's realized early growth as an indicator of future production potential. Genetic information of the dam and sire is included to reflect the genetic potential of the calf. Scientific evidence consistently demonstrates that early growth rate is strongly associated with lifetime productivity in cattle, supporting the use of age-adjusted growth indicators to predict future performance. Model development integrates scientific research and production data. Growth curves and expected feed intake were based on controlled trials of Beef on Dairy and purebred bull calves conducted at Aarhus University. These curves are scaled to fit production levels on all animals slaughtered in Denmark the past two years. Likewise the, calculating expected breed level of dressing percentage, EUROP score and mortality was extracted based on the same data. Financial records and abattoir pricing are used to estimate production costs and are regularly updated to reflect market conditions. The model predicts carcass weight with a root mean squared error (RMSE) of 24 kg and net growth with an RMSE of 155 g/day. While prediction error is non-negligible, it reflects variation observed in practice and compares actual carcass weight to expected carcass weight at day 300. As an illustration, the price difference between 24-day-old Beef on Dairy calves at the 25th and 75th percentile in weight is approximately 138 €. The model introduces a calf-specific base price and distributes the expected margin between dairy producer and finisher according to predicted performance levels. Stakeholder representatives have decided on a distribution model of expected revenue, making a fair price distribution, while creating incentive to increase early life growth of the calves. Market adjustment of the price enable alignment with supply–demand conditions without obscuring biological foundation for the model. The approach has implications for breeding strategies, use of sexed beef semen, colostrum management, and nutritional management during the neonatal period. By making the economic consequences of suboptimal early management more explicit, the model aims to strengthen incentives to improve calf robustness and uniformity. In practice this means that there can be up to 135 € difference in estimated value for calves of the same sex, breed combination and weight purely due to their age at the time of sale. The price difference is justified by a higher income potential of the calf. The topic concerns Beef on Dairy production systems and integrates principles from animal breeding, production economics, and herd management. Relevant stakeholders include dairy producers, calf finishers, breeding organizations and advisory services. Beyond economic efficiency, improved alignment of incentives across the value chain may contribute to improved animal welfare outcomes and societal acceptance of dairy-origin beef production.

HerdPlanner: Decision models for reproduction and culling decisions

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Farm size is increasingly constrained by physical limitations (e.g., number of cubicles) and environmental regulations (e.g., nitrogen, phosphate, or CO_2 emission limits). To sustain profitability under these constraints, farmers must strategically balance youngstock numbers with the population of lactating cows. This is challenging because insemination decisions affect herd composition only after a delay of nearly three years, when resulting heifers enter their first lactation. HerdPlanner supports this decision-making process by guiding optimal insemination and culling strategies at the individual-cow and herd levels. Three workshops involving 29 farmers from across The Netherlands and Flanders were conducted to identify key challenges related to insemination choices, voluntary and forced culling, and long-term herd composition. We used their feedback to define a phased development roadmap for HerdPlanner. Phase one focuses on insemination advice. At the individual-cow level, decision models recommend to inseminate, wait, or not inseminate, using expected performance, lactation stage, projected dry-off yield, health status, and genetic merit. At the herd level, the models advise how many inseminations with sexed, conventional, or beef-on-dairy semen should be performed per 21-day cycle to achieve the desired number of replacement heifers for herd stability. These advisory functions are provided through an experimental module, "AI Lab", within CRV's farm management software. AI Lab enables rapid iteration based on user feedback regarding both content and presentation of model results. Phase two will expand the tool with culling recommendations informed by expected individual-cow economic performance. The advisory functions of Phase One are currently being evaluated through qualitative and quantitative feedback collected from approximately 50 farms over several months. In this presentation we will share and discuss the functionality and the insights we derived from the collected feedback. We will use that feedback to further improve HerdPlanner so that it provides farmers with actionable, model-based decision support to optimize herd composition within physical and environmental boundaries.

Fattening and slaughter performance of crossbred, German Holstein and Simmental bulls fed with sweet white lupine as protein source

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The practice of mating beef sires on German Holstein (HF) dams is increasing in several countries. Crossbreds from beef on dairy mating have a higher economic value because of heavier carcasses and lower proportions of trim than dairy bulls. Furthermore, interest in marketing regionally produced quality meat, made predominantly from locally produced feed, is increasing among both producers and food retailers. The various crossbreeds pose challenges, especially for fatteners, such as knowledge gaps concerning growth patterns as well as fattening and slaughter performance. Differences in nutrient and energy requirements of crossbreeds are currently given little consideration in ration calculations and feeding. Findings in this regard are of great importance for optimizing fattening and improving the economic efficiency of fattening farms. Therefore, the aim of this study is to investigate growth patterns as well as fattening and slaughter performance of regionally relevant crossbreedings. In addition, the suitability of sweet white lupine (SWL, lupinus albus) as a concentrated feed component in bull fattening will be tested. This will highlight an option for using a regional and sustainable protein feed in place of soybean meal. The trial included 150 bulls of HF (n=49), Simmental (FV, n=21), Angus x HF (ANGxHF, n=37), and Uckermärker x HF (UCKxHF, n=43). The 150 bulls were divided into two feeding groups. Both groups received an identical basic ration consisting of corn silage, grass silage, straw, pressed sugar beet pulp, wet corn, and mineral feed. In the control group (CG, n=79), protein supplementation was provided via soybean meal, whereas in the lupine group (LG, n=71), it was provided via SWL and soybean meal in the pre-fattening phase and only SWL in the final fattening phase. When optimizing the total rations, crude protein is equated. For each group, feed consumption and feed costs are recorded. The bulls are weighed five times during the trial period (after birth, day 28, day 90, day 185, day 325). In addition to the weighings on days 185 and 325, the longissimus dorsi muscle area and fat thickness of the animals are also measured using ultrasound. After birth calves weighed 42.6 kg (HF), 45.5 kg (ANGxHF) and 52.5 kg (UCKxHF). For the FV birth weight was not available. The trial started in February 2025 with an average age of 6.2 months and a weight of 247 kg. At that point highest back muscle area was shown by UCKxHF with 57.1 cm² and lowest by HF with 43.4 cm². The ANGxHF bulls had the highest fat thickness (3.13 mm) at start of trial, lowest UCKxHF (2.41 mm). In the pre-fattening phase the average daily weight gain (ADG) was higher in the LG (1,438 g/day) than in the CG (1,337 g/day). Furthermore, the LG showed a higher feed intake with 7.9 kg DM/d per animal than CG (7.6 kg DM/d). Highest ADG was observed in crossbred UCKxHF (1,469 g/day). The bulls were slaughtered in March 2026. To analyse differences between genotypes and feeding groups, parameters like carcass weight, slaughter yield, carcass conformation score, leg circumference, intramuscular fat and meat tenderness are recorded. More comprehensive results on slaughter performance and carcass quality will be presented and discussed at the conference. This study provides new insights on the suitability of SWL as a regional ration component for bull fattening. Moreover, internationally relevant and economically decisive recommendations can be given regarding the selection of suitable breeds or crossbreeds.

Post-weaning feed management of Holstein and beef × Holstein cattle: Effects on growth performance and carcass merit

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Optimizing post-weaning nutritional strategies for beef × Holstein (B×H) cattle is critical to maximize growth efficiency and carcass value while minimizing health risk. We hypothesized that a moderate-energy growing program would improve retail cut yield and that B×H cattle would have improved growth and carcass traits over Holstein steers. The effects of dietary energy density during the growing phase on intake, growth performance, gain-to-feed ratio (G:F), and carcass merit of Holstein steers, B×H steers, and B×H heifers were evaluated. Weaned calves (n = 40/breed-sex; 205 ± 23 kg body weight, BW) were randomly assigned to either a rapid gain (RG; 1.9 kg/d) or moderate gain (MG; 1.2 kg/d) program during the growing phase (n = 20/breed-sex × program) and individually housed. The RG and MG grower diets contained 48% and 28% starch, respectively, and 15% crude protein (CP). When 70% of each breed-sex × program group reached 350 kg BW, cattle were transitioned to a common finishing diet (51.0% starch, 13.5% CP) and fed to market weight (steers, 756 ± 62 kg; heifers, 703 ± 41 kg). Intake was measured weekly and summed over 28 d periods to calculate gain-to-feed ratio, while BW and body measurements were obtained every 28 d. Continuous data were analyzed using PROC MIXED in SAS (Cary, NC, USA) with breed-sex, program, and the 2-way interaction as fixed effects, and barn wing as a random effect. Initial BW was a covariate for performance data, while final BW was a covariate for carcass data. Carcass quality grade (QG), yield class (YC), and liver abscess score (LAS) were analyzed using PROC GLIMMIX with a multinomial distribution and weighted count variable. No significant ($P > 0.05$) breed-sex × program interactions were detected. Dry matter intake did not differ by program, but was greater ($P < 0.01$) for B×H steers than Holstein steers and B×H heifers. Average daily gain (ADG) was greater ($P = 0.03$) for RG cattle than MG, and greatest ($P < 0.01$) for B×H steers. Gain-to-feed was unaffected by program, but was greatest ($P < 0.01$) for B×H steers and lowest for Holstein steers. Hot carcass weight (HCW) did not differ by program; however, B×H steers had greater ($P < 0.01$) HCW than Holstein steers and B×H heifers. Cattle fed RG had greater ($P < 0.01$) marbling score and backfat-thickness than MG cattle. Backfat-thickness differed among breed-sex ($P < 0.01$) with B×H heifers having the greatest and Holstein steers having least. Breed-sex and program did not influence ($P > 0.05$) QG or LAS, whereas YC differed by breed-sex and program ($P < 0.01$). The B×H steers were more likely (odds ratio, OR = 0.20; $P < 0.01$) to have higher carcass YC and tended toward lower odds (OR = 0.39; $P = 0.07$) of severe LAS than Holstein steers. The RG cattle had greater odds (OR = 7.55; $P < 0.01$) of poorer carcass YC than MG cattle. In summary, targeting RG during the growing phase improved ADG and marbling score without affecting gain-to-feed or liver health and did not interact with breed-sex. However, RG increased the likelihood of poorer carcass yield class, partially supporting our hypothesis and highlighting trade-offs between carcass quality and cutability in beef × dairy systems.

Effect of the inclusion of cocoa bean shells in the diet of fattening beef on live performance, carcass traits, and nutritional quality of the meat

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Cocoa bean shells (CBS) represent a sustainable by-product for ruminant diets, but their long-term effects on local breeds remain underexplored. Aim of this study was to evaluate the impact of CBS inclusion on performance, blood parameters, carcass traits, and meat quality in Sardo Bruna cattle. Thirty Sardo Bruna cattle (15 females, 15 males) were divided into 3 groups (n = 10 per group) balanced by sex: control (CTR, basal diet), low-dose CBS (LD, +150 g/head/day), and high-dose CBS (HD, +300 g/head/day). The basal diet was offered ad libitum and was composed of a base of forage and concentrate mixed with a feed mixer offered twice daily. The forage component included straw and hay, while the concentrate included a commercial concentrate and corn meal. Over 6 months, monthly BW was recorded and average daily gain (ADG) was calculated. At slaughter, carcass weight and yield were measured; pH, temperature, and color (CIE Lab*) were assessed on Longissimus thoracis et lumborum (LTL) and Rectus abdominis muscles. LTL samples were analyzed for proximate composition; fatty acids profile and cooking and drip losses. Data were analyzed by ANOVA with diet, sex, and their interaction.Neither BW nor ADG differed by diet and males showed higher ADG than females ($P<0.05$). Carcass yield and cut weights were unaffected by CBS supplementation. In Rectus abdominis, HD increased b* parameter compared to CTR and LD $P<0.05$); females showed higher a* and b* in LTL muscle ($P<0.05$). Drip and cooking losses were unchanged by diet. Proximate composition was similar across diets, but HD increased PUFA n-3 in LTL muscle compared to CTR and LD ($P<0.05$). At the same time, sex influenced the FA profile: females had higher MUFA and males higher PUFA ($p<0.05$). Long-term CBS supplementation sustains live performance and carcass traits in Sardo-Bruna cattle while enhancing meat PUFA n-3 content. Overall, the study supports the safe use of CBS as a sustainable dietary supplement without compromising animal performance and meat quality. Acknowledgements: this work was supported by the University of Sassari Interdisciplinary Research Project (DM 737/2021, 2021-2022 funds), funded by the European Union – NextGenerationEU.

An integer programming approach to optimal selection and mating for herd improvement

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Sustained genetic progress in dairy herds requires transparent, data-driven guidance that supports both farmers and breeding advisors. We present an integrated framework that delivers optimized selection and mating recommendations by balancing genetic gain, inbreeding management, and farm-specific breeding objectives. The approach explicitly incorporates operational realities at the herd level, ensuring that genetic progress aligns with practical constraints. Methods The framework is built around a two-stage integer linear programming (ILP) procedure. Inputs include genomic breeding values, pedigree-derived inbreeding coefficients, recessive defect carrier status, trait-specific economic weights, and a comprehensive set of herd-level operational constraints. These constraints cover semen allocation rules, bull usage limits, prioritization of on-farm semen inventory, and parity-specific calving-ease conditions. For each potential mating, expected genetic merit, inbreeding, and defect-risk metrics are calculated. The first ILP identifies the mating plan that maximizes overall herd genetic gain while satisfying biological and management constraints. A subsequent optimization step enhances conformation consistency across the herd, providing coherent selection and mating guidance within a unified decision-support framework. The system was evaluated on 33 commercial dairy herds in the Netherlands, generating recommendations in parallel with conventional expert-driven breeding advice. Predicted genetic progress, compliance with farm constraints, and user feedback were compared between approaches. Farmers and advisors also completed a qualitative survey regarding clarity, perceived usefulness, and alignment with breeding goals. Results and conclusions Across all participating herds, the optimization framework delivered predicted genetic gains that matched or exceeded those from standard advisory procedures while fully respecting operational and biological constraints. Survey responses from both farmers and breeding specialists indicated strong overall acceptance, highlighting the clarity, practicality, and goal alignment of the recommendations. These results demonstrate that data-driven optimization can strengthen on-farm genetic decision-making and offer a scalable approach for future herd improvement tools.

Characterizing the complex genetic architecture of SINS reveals associations with physiological side effects of selection in swine

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Background and Objectives Swine Inflammation and Necrosis Syndrome (SINS) is a clinically defined endogenous syndrome in pigs, manifesting as lesions of tail, ears, teats, coronary bands, heels, and other body parts. It is widely distributed across countries and provides an animal-based measure to reduce tail lesions during fattening and at slaughter, a core welfare indicator. The aim of this presentation is to review the interactions between clinical, clinical-chemical, pathohistological, metabolomic, transcriptomic, and genomic data, and their implications for breeding and genetics. Genetic architecture of SINS While environmental factors such as feeding, management, and housing contribute, our data demonstrate that susceptibility to SINS is strongly genetically determined. Transcriptional profiling shows that clinical SINS is associated with upregulation of inflammatory and acute-phase genes and liver metabolic reprogramming, indicating a systemic rather than localized response. Genomic analyses reveal that key mediators of these pathways are heritable and distributed across multiple quantitative trait loci, establishing a polygenic architecture underlying individual susceptibility. SINS correlates with physiological traits commonly observed as side effects of intensive selection for performance, including metabolic stress, immune dysregulation, and reduced resilience. Modern breeding increasingly includes tail integrity as a performance trait, economically incentivized in production. Because SINS predisposition directly affects tail health, selection for intact tails intersects with genetic susceptibility to SINS, highlighting a practical limitation and the tension between production goals and animal welfare. Application in breeding programs These findings illustrate that expression of genetic potential in high-performance pigs can produce unintended consequences for health and welfare. SINS serves as a model where clinical manifestation, genetic architecture, and physiological correlates converge, emphasizing opportunities and constraints in modern swine breeding. Integrating clinical, transcriptomic, and genomic evidence shows that SINS should be considered both a heritable syndrome and a phenotypic indicator of physiological stress, rather than solely an environmental problem. These insights inform multi-trait selection strategies that balance growth, feed efficiency, reproductive performance, tail integrity, and resilience, providing a framework to manage correlated physiological risks in production.

Genetics of swine inflammation and necrosis syndrome: What do we know so far?

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Skin lesions in pigs are commonly attributed to damaging behaviors such as tail and ear biting and represent major welfare and economic concerns in swine production. However, lesions observed at birth or shortly thereafter suggest that some injuries may originate from Swine Inflammation and Necrosis Syndrome (SINS), a condition characterized by inflammatory and necrotic alterations in the extremities, including ears, tails, teats, and claws, which can be observed at birth, before animal interaction, or other factors such as flooring or tail docking. Across studies conducted within Topigs Norsvin, involving different pig lines and breeds, results indicate that SINS has a genetic component, with low-to-moderate heritability estimates (0.03–0.34). Both direct genetic effects and smaller maternal effects contributed to variation in the syndrome. A consistent finding across these studies is a genetic association between early-life SINS, observed in piglets up to 3 days of age, and skin damage observed in finishers, with genetic correlations ranging from 0.19 to 0.78. Wide ranges in heritability and genetic correlation are due to variation among breeds, lines, trait definitions, and body regions evaluated. The genetic relationship between SINS and skin damage in finishers suggests two possibilities: (1) pigs more prone to inflammatory lesions early in life may also be more susceptible to injuries often attributed to damaging social behaviors, and (2) skin damage observed in the finishing stages, commonly associated with abnormal behaviors such as ear and tail biting, may be partially confounded with SINS. In either case, selection against SINS could indirectly reduce the prevalence of skin damage in finishers. Beyond the relationship between SINS and skin damage, SINS was also genetically correlated with production traits such as birth and weaning weight (-0.40 to -0.30), whereas it showed only minor relationships with carcass traits such as loin depth and backfat (-0.16 to 0.05). Although these findings highlight the genetic relevance of SINS and its potential role as an early indicator of welfare outcomes, its relationships with other production and reproduction traits remain to be further explored. Therefore, this study aims to investigate the genetic relationships among SINS, reproductive traits, and cardiorespiratory traits assessed via CT scanning in a purebred dam line. The SINS dataset includes binary information on lesions of teats, ears, and tails, recorded within the first three days of life for 6,229 piglets born between 2022 and 2025 across one Canadian and two Dutch farms. For a subset of these animals and their relatives across up to three generations, reproductive performance (N = 14,717) and CT-derived cardiorespiratory traits (N = 13,069) were available. Reproductive traits included total number born, number of stillborn, gestation length, and litter mortality, while CT scan traits captured heart and lung weights. We hypothesized that SINS would exhibit weakly unfavorable genetic correlations with reproductive traits and a moderately unfavorable correlation with cardiovascular capacity, supporting previously reported links between SINS and blood flow and circulatory disturbances.

Ear necrosis: Evidence of pigs reaching their biological limits?

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Ear necrosis has emerged as a major welfare problem arising from the intensification of pig production. It is a prevalent but poorly understood, multifactorial disease affecting young pigs in intensive systems worldwide, and there are currently no successful mitigation strategies. Through a series of experimental and observational studies across 4 years, we examined the prevalence and progression of ear necrosis, and relationships between ear necrosis and several factors including early life experiences, management practices, damaging and aggressive behaviours, and physiological biomarkers. The research confirmed that ear necrosis development cannot be attributed to any single factor. Controlled trials were conducted to investigate the influence of pre- and post-weaning supplementary nutrition strategies and pre- and post-weaning improved hygiene protocols on post-weaning ear necrosis development. Despite some positive effects on pig growth and feed intake, there was no effect on ear necrosis ($P > 0.05$), indicating that single-variable interventions are unlikely to successfully ameliorate the disease. Additionally, we found the first conclusive evidence that ear biting behaviour performed by piglets ($N = 298$) during the pre-weaning period has a critical influence on the future risk of ear necrosis: an increase in the frequency of ear bites received at week 3 of age increased the risk of developing ear necrosis post-weaning ($OR = 3.34$; $P < 0.05$). Histopathological examination of ears from 32-day old piglets revealed that pre-weaning ear biting behaviour causes measurable tissue damage, supporting the hypothesis that repetitive oral manipulation compromises tissue integrity and increases the risk of ear necrosis. An investigation of physiological biomarkers in oral fluid demonstrated that ear necrosis is associated with elevated markers of inflammation and immune activation. A trial conducted on a commercial grower-finisher farm ($N = 48$ pens; 1,676 pigs) revealed a high prevalence of ear necrosis and negative behaviours (ear, tail, and flank biting, and aggression), and considerable pen-level variation, suggesting the presence of pen and individual-level risk factors. A study of 21 commercial Irish farms confirmed the widespread nature of ear necrosis: ear necrosis was found on 100% of the farms and an average of 30% of pigs were affected per farm, which is an increase from the reported prevalence 7 years prior. Ear biting behaviour was performed at high levels throughout the production stages across all studies. Together, this research contributes novel evidence regarding ear necrosis aetiology, emphasizing the complex, context-dependent nature of risk factors, where ear biting behaviour plays a key role. The research suggests that ear necrosis is a production disease, i.e. the undesirable outcome of increasing production pressures rendering pigs unable to cope. In conclusion, this underscores the difficulties of addressing the disease within the constraints of the current production system.

Pathological investigation of cardiovascular disease in broilers

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This abstract addresses cardiovascular disorders in broilers, presented as a case example for the session's structured discussion. Cardiovascular problems, often associated with rapid growth, remain the most common cause of mortality in broilers under non-outbreak conditions and are a major reason for carcass rejection at slaughter. Evidence suggests a genetic contribution, and pathology indicates two forms: an acute form characterised by generalised circulatory disturbances, oedema and congestion, and a chronic form involving cardiac dilatation, liver cirrhosis and ascites. This study included Ross 308 broilers at 33 and 41 kg/m² (slaughtered at 34 days) and Rustic Gold broilers at 36 kg/m² (slaughtered at 41 days) and used a repeated cross-sectional design (3 rotations/group) across 33 flocks on eight Danish farms (2023–2024). From day-old to slaughter, dead, euthanised or rejected birds were collected and necropsied. Of 6839 birds, 3503 birds (51%) were classified with cardiovascular collapse, each showing ≥3 characteristic lesions. First-week mortality, cumulative mortality, dead on arrival (DOA) and carcass rejection rates were within normal range in all flocks and did not differ significantly between broiler groups. During the first week, cardiovascular collapse accounted for 28.7% of deaths, with no significant differences between broiler groups. Ventricular dilatation occurred in 8.7% of 1-day-old chicks but was rarely accompanied by other cardiovascular lesions, suggesting genetic or hatchery-related factors. From day 8 to slaughter, cardiovascular collapse was the dominant cause of on-farm mortality (81%). Overall cardiovascular collapse rates did not differ significantly between broiler groups, but both Ross 308 broiler groups had significantly higher rates of cardiac dilatation and ascites than Rustic Gold. Birds >28 days were more likely than younger birds to exhibit chronic lesions such as ascites and liver cirrhosis. Distinct lesion patterns allowed differentiation between chronic heart failure and Sudden Death Syndrome (SDS), the latter characterised by acute circulatory disturbances and lung oedema. Among DOA birds, 75% were classified with cardiovascular collapse, with significantly fewer birds involving cardiac dilatation in Rustic Gold. Low body weight (<1750 g) was associated with higher rates of ascites, cardiac dilatation and liver cirrhosis in both DOA and rejected birds, indicating prolonged survival of chronic cases. Neither the use of Rustic Gold birds nor lowering stocking density reduced cardiovascular-related DOA outcomes. Ascites, the third most common cause of carcass rejection (17.2%), occurred more frequently in Ross 308 birds than in Rustic Gold. The probability of chronic cardiovascular lesions was doubled in carcasses <1.750 g. Overall, cardiovascular disorders accounted for 51% of deaths and rejections across all stages despite normal flock mortality rates. Genetics, management and environment have previously been shown to influence susceptibility on both chronic heart failure and SDS. Findings highlight a substantial welfare burden and emphasise the need for integrated breeding and management strategies that balance welfare improvements with economically sustainable production. This raises the question: what is an acceptable level of cardiovascular disease in broiler production?

From behavior to immunity: Sex-related differences in immune cell composition in chicken lines selected for high and low feather pecking

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Feather pecking is a serious behavioural disorder in chickens, leading to feather loss, skin injuries, increased stress, and higher mortality rates. Despite extensive research, the underlying mechanisms are not fully understood. Feather pecking is considered a multifactorial trait influenced by genetic, environmental and physiological factors, including the immune system. It has a higher incidence in hens compared to roosters. Studies in humans and mice have demonstrated that psychiatric disorders and related personality traits, such as impulsiveness and hyperactivity, are associated with alterations in the immune system, particularly in T-cell composition and inflammatory processes. Given common features between FP in chickens and humans with psychiatric disorders, feather pecking may likewise be associated with alterations in T cell immunity. The aim of this study was to investigate the immune system of hens and roosters of a high (HFP) and low feather pecking (LFP) line to identify differences between genetic predisposition and sex. Lines had been divergently selected based on the severe feather pecking behaviour of hens, resulting in a HFP and a LFP line. A total of 120 hens (60 HFP, 60 LFP) and 60 roosters (30 HFP, 30 LFP) from the 20th generation of selection were examined. At 42 to 43 wk of age, birds were slaughtered, and blood, spleen and caecal tonsils were sampled. Immune cell populations were characterized using flow cytometric analysis using a no-lyse-no-wash method and staining with chicken-specific fluorochrome-conjugated antibodies. Statistical analyses were performed using a linear mixed model (SAS 9.4). Immunological differences were observed both between sexes and between selection lines, with several effects being also tissue-specific. Overall, roosters had higher numbers of several immune cell types in spleen and caecal tonsils compared to hens. In the spleen, higher cell numbers in roosters were mainly attributable to their larger organ mass, whereas in the caecal tonsils they primarily reflected higher cell density. In spleen and blood, HFP chickens had generally lower immune cell numbers than LFP chickens, indicating genetic differences in immune cell availability or mobilization. Line effects in the caecal tonsils were predominantly sex-dependent, particularly affecting $CD4^+$ cells and their subsets. Notably, $CD4^+$ cell numbers were exclusively lower in HFP hens. These data demonstrate an association between immunity and feather pecking and suggest that divergent selection for feather pecking influenced immune cell profiles. Given that feather pecking is predominantly expressed in females, the selective reduction of $CD4^+$ cells in the caecal tonsils in HFP hens supports the hypothesis that feather pecking might be an immune-associated behaviour, potentially linked to immune dysregulation in the gut.

Investigating differential gene expression in the pectoral muscles of broiler chickens affected by wooden breast condition

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Nowadays, poultry represents the primary driver in global meat production. This has been possible due to the remarkable advancements in poultry selection, nutrition, housing, and meat processing. As a downside, the outstanding progress in this field also determined the occurrence of challenges. Among these, the manifestation of meat quality defects, commonly referred to as "growth-related myopathies", particularly affecting the Pectoralis major muscles (PMs) of the modern fast-growing broilers, remains a major area of research. Within this context, the present study was developed within the PRIN 2022 PACHOL4 project, which aims to investigate the molecular basis of wooden breast (WB), a major quality defect in broilers, with a specific focus on characterizing the transcriptomic profile of WB-affected breast muscles. Given the importance of accurately classifying PMs to properly investigate factors associated with the onset of these abnormalities and to avoid misleading results, a total of 180 breast fillets were collected at a commercial processing plant and classified as WB-affected or normal (N) based on their macroscopic appearance. After validating their macroscopic classification through multivariate analysis of meat quality data, 10 samples/group (WB and N) were selected and used for transcriptomic analysis. Briefly, total RNA extracted from each sample was checked for quality and used for RNA-seq using NovaSeq X-Plus technology. Raw-reads quality check, trimming, alignment to the reference genome, and count were performed on the High-Performance Computing platform provided by ELIXIR-IIB HPC@CINECA. After raw-reads processing, differential analysis was performed in R using DESeq2 and limma packages, while functional categories significantly enriched by the DEGs were detected by using DAVID Knowledgebase v2024q4 and considering a Benjamini–Hochberg adjusted p-value of 0.05 as significance threshold. Considering a log2fold-change>|1| and a q value<0.05, a total of 565 differentially expressed genes (DEGs) were found. Among those, 483 and 82 were respectively up- and down-regulated in WB compared to N. In accordance with the literature, functional analysis of up-regulated DEGs evidenced genes involved in PI3K-Akt and cytokine-mediated signaling pathways (P<0.001), as well as cellular components related to collagen trimer composition and ECM-receptor interaction (P<0.001), while functional terms related to carbon metabolism, glycolysis, and biosynthesis of amino acids were found enriched by the down-regulated DEGs (P<0.0001). Notably, MYBPC3, TNNT2, and ACTC1, which are involved in the dilated and hypertrophic cardiomyopathies in humans and are generally transiently expressed in embryonic chick skeletal muscles, were found among the up-regulated DEGs in WB (P<0.05). Therefore, the present results not only corroborate part of the existing literature but also highlight the dysregulation of several genes involved in cardiac-related pathways in PMs affected by WB. Even though a direct association with cardiac hypertrophy in WB-affected birds cannot be speculated, similarities in the underlying biological and molecular mechanisms between these two conditions, as well as the occurrence of gene expression patterns resembling the early stage of muscle development in WB muscles, have been hypothesized. This research was supported by NextGenerationEU, National Grant PRIN2022 (Prot. n. 2022EPWEPW).

Mapping production independent mastitis risk in German Holsteins using GWAS SEM

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Mastitis is among the most costly diseases in dairy cattle worldwide. Its antagonistic genetic correlation with milk production traits poses challenges for reducing mastitis risk in high-producing animals without compromising production performance. Thus, we aimed to separate mastitis specific genetic signals from those shared with milk yield using structural equation modeling (SEM) and to integrate gene expression data to identify possible causal genes underlying mastitis risk independent from milk yield. The relationship between the single nucleotide polymorphisms (SNPs; n = 16,882,734) and the traits milk yield and mastitis was captured by a Cholesky model. Two mediating latent factors were defined that exclusively impacted mastitis phenotypes or affected both milk yield and mastitis. Regression weights of the latent factors on the observed traits were determined using heritabilities and genetic correlations of milk yield and mastitis estimated in 34,497 German Holstein cows. Based on GWAS summary statistics for milk yield and mastitis, SNP effects on the latent factors were then estimated to identify loci associated with production-independent mastitis susceptibility. A two-sided Z-test was performed to identify genome-wide ($p < 2.96*10^{-9}$) and suggestively significant SNPs ($p < 5*10^{-6}$). Finally, we intersected publicly available gene expression data on single-cell level (8,866 bulk RNA-seq samples plus 999,192 single cells) with those loci. We found that 91 % of the genetic variance in mastitis was production independent. Although no SNPs passed Bonferroni-correction ($p < 2.96*10^{-9}$), 922 SNPs were suggestively significant for production-independent mastitis risk ($p < 5*10^{-6}$). Of these, 77 % of hits were located within the GC-NPFFR2 region on BTA6. The lead SNP was located on BTA10, closest to SEMA6D located 251 kb downstream. The strongest candidate gene based on the integration of expression QTL (eQTL) data was GRIP1 (BTA5) occurring in several layers of the single-cell resolution eQTL data and displaying consistent effect directions within layers. Our findings are consistent with known candidate genes for mastitis resistance in dairy cattle. The identified genes are involved in cytokine signaling, macrophage activation, and inflammation, and are plausible in the context of mastitis resistance. Collectively, this study shows that SEM combined with the integration of gene expression data is a promising tool for disentangling the interrelationships of antagonistically correlated quantitative traits. The identified candidate genes could help to design future breeding schemes for healthy and high producing animals.

From genetic potential to robustness: insights from energy-allocation models

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The mismatch between animal genetics and production environments is a long-standing concern that may become increasingly prominent under future farming conditions. Classical quantitative-genetic analyses often reveal limited genotype-by-environment interactions (G × E), yet physiological evidence suggests that biological trade-offs can affect relationships between traits when animals face nutrient scarcity or high metabolic demands. Such constraints on resource allocation are thought to play a central role in animal robustness. Within this framework, energy-allocation models integrating quantitative genetics have been proposed to explore how G × E may emerge from underlying physiological processes. Based on published modelling studies and on our own work with a dynamic sheep model, we discuss the potential benefits and limitations of this approach. A key assumption is that input coefficients controlling energy allocation among maintenance, growth, reproduction, and body reserves are genetically variable and less sensitive to environmental variation than model outputs representing observable performance traits. Although this assumption remains untested, it is key for simulating animal responses across diverse conditions. Simulation experiments show that genetic variation in specific allocation coefficients can generate substantial differences in lifetime trajectories and help identify robust animals. We also show that the assumed genetic variances and covariance structure among input coefficients strongly influence the genetic parameters of output traits and must therefore be chosen carefully. Finally, combining mechanistic modelling with a breeding-scheme simulation highlights differences between predicted selection responses and those obtained with classical quantitative genetics. Overall, we provide a balanced perspective on the use of mechanistic models in a genetic context: their complexity allows to reveal key biological insights into G × E but it also poses strong challenges for empirical validation.

From genotype to phenotype: expectations and limitations

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Once the oocyte and sperm are united forming the embryo, genetic potential drives the development of the new organism. However, during embryonal and fetal genesis in utero or in ovo, maternal or environmental challenges modulate the genetic potential by epigenetic modifications with consequences for later life. After birth or hatch, a second plastic window during early life allows further long-lasting epigenetic modifications. After weaning or hatch, high performing farm animals are fed according to their genetically determined growth potential and high feed intake capacity. Thus, production of meat is highly effective and cost-efficient. However, rapid growth can lead to immature tissues and organs and may thus not be efficient. By intensive postweaning feeding, female farm animals are pushed to reach their sexual maturity as early as possible. Immaturity of tissues and organs in females could cause a weaker fertility and shorter productive lifespans. The genetic selection for specific metabolic performances such as milk and eggs promotes extensive growth and development of target organs which leads to disproportionality between anatomical and functional features within the individual. Selective breeding for performance has set an imbalance within the orchestrated interplay of all organs and tissues. Because of immaturity and disproportionality, animals develop a high risk to suffer from production diseases and reduced resilience. Therefore, it is often observed that the phenotype predicted by the genotype is not matching the phenotype in production reality due to epigenetic modulations, rearing and feeding regimens and management decisions. The presentation will show an overview about current knowledge of physiological processes and their plasticity in ontogenesis of individuals which may help to explain the mismatch between genotype and phenotype. Respective examples in different farm animal species will be presented in detail in the talk.

Balanced breeding goals: genetics as part of the solution to livestock welfare and sustainability challenges

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Selective livestock breeding has delivered remarkable gains in productivity over the past decades, enabling the efficient production of high-quality animal protein for a growing global population. As the demand for animal-source foods increases, livestock systems must simultaneously improve productivity, reduce environmental impacts, and maintain high standards of animal health and welfare. This challenge has intensified the debate about whether modern breeding has pushed animals beyond their biological limits. These concerns are legitimate. For example, high-performing dairy cattle populations have faced challenges related to metabolic stress, fertility, and longevity, while in poultry, skeletal disorders and breast muscle myopathies raise similar questions about the biological consequences of rapid genetic progress. Such observations highlight the need to continually reassess breeding goals as biological insights, production environments, and societal expectations evolve. Over the past two decades, livestock breeding has fundamentally shifted towards multi-trait selection frameworks that integrate productivity with health, welfare, robustness and functional longevity. Where early breeding programs prioritized yield and risked unfavourable correlated responses in functional traits, contemporary breeding programs increasingly incorporate health, fertility, longevity, efficiency, and resilience directly into multi-trait selection indices. Evidence across species show these balanced breeding goals enable simultaneous genetic progress in productivity and functional traits, demonstrating that genetic improvement can actively contribute to healthier and more robust animals. Advances in quantitative genetics and genomics have enabled this shift. Large-scale phenotyping and genomic evaluations now allow complex welfare-related traits to be measured and improved through selection. In dairy cattle, genetic studies have demonstrated heritable variation for mastitis resistance, fertility, longevity, metabolic stability, and resilience to environmental stressors such as heat stress. In poultry, research has identified genetic components underlying leg health disorders and muscle myopathies, providing the basis for breeding strategies that reduce their incidence while maintaining production efficiency. Genomic tools have also transformed the management of rare but severe genetic defects. Routine genomic screening enables the identification of carriers of deleterious variants, allowing breeders to avoid high-risk matings and substantially reduce the prevalence of harmful alleles without compromising genetic progress. Importantly, genetic improvement is increasingly aligned with broader sustainability goals. Selection now addresses feed efficiency, methane emissions, resilience to climatic stressors, and overall robustness, features that help animals maintain health and performance across variable environments and production systems. Feeding a growing global population while reducing the environmental footprint of agriculture requires animals that are productive, healthy, resilient, and efficient. Genetic improvement alone will not solve the complex challenges facing livestock systems, but, when integrated with advances in nutrition, management, and veterinary care, it remains one of the most powerful and durable levers for improving the welfare, sustainability, and productivity of farm animals.

Why laying hens struggle with vertical space: biomechanical constraints and implications for aviary design

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Aviaries are designed to promote freedom of movement, yet laying hens face substantial biomechanical challenges when navigating vertical space. Modern hens are ground-dwelling, high-wing-loading birds whose flight consists of short, explosive bursts rather than controlled aerial manoeuvring. When key resources are located on upper tiers, hens must rely on descent by flapping or gliding, but their capacity to adjust trajectory, decelerate, or modulate lift appears limited. Understanding these constraints is essential for designing housing systems that align with the physical abilities of today's strains to avoid injuries. To examine these limitations, we synthesized findings from three biomechanical studies (Leon et al., 2021; van Staaveren et al., 2023; Hong et al., 2024) involving hens of different ages (34–70 weeks), strains, health conditions, and training histories. Across studies, we quantified flight kinematics and ground reaction forces during descent and landing. In the first study, feather clipping reduced wing area in 34-week-old Lohmann LSL Lite hens (n=18) to challenge aerodynamic capacity. Hens descending from 1.5 m with ~50% wing area exhibited rapid vertical velocity (3.9 m/s) and high horizontal acceleration, without compensatory changes in wingbeat frequency, amplitude, or angular velocity, indicating that hens operate near their muscle power limits and cannot adjust wing control when lift is compromised. A second study examined the influence of painful conditions—foot pad dermatitis (FPD) and keel bone fracture (KBF)—on landing mechanics in 70-week-old hens (n=37). Landing velocity and impact force increased with height (3.3× body weight at 30 cm; ~6.6–7.1× BW at 170 cm). Hens with KBF showed higher descent velocities (+17–21%) and tended to experience higher peak landing forces (+24%) than those with FPD. Meloxicam did not reduce landing loads and, in hens with FPD, tended to increase velocity and impact (~+16% BW at 170 cm). A third study evaluated whether 16 weeks of wing-assisted incline running (WAIR) training improved flight performance. In 37-week-old hens (n=82), WAIR modestly increased initial flight velocity (+6.5%) and tended to reduce descent acceleration, but descent velocity and trajectory angle (33–37°) remained unaffected. Collectively, these studies demonstrate that laying hens possess limited biomechanical capacity to modulate descent, require substantial landing distances, and experience high impact forces—constraints intensified by feather loss, injury, or reduced lift. As aviaries are complex, these physical limits highlight an urgent need for housing systems that match the abilities of modern laying hens, rather than assuming birds can adapt to environments that exceed their biomechanical thresholds.

When genetic potential meets production reality: implications for the animal. Discussion and summary of the session

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This seminar should summarize implications for the animal regarding its genetic potential, its resulting phenotype in production reality and associated changes in behavior. The final phenotype of a farm animal is based on genotype but also on many other influencing factors such as management and feeding which could lead to changed behaviors. Many questions arise from this interrelationship which will be discussed in this session´s seminar: Could we improve genotype-phenotype relationships and thereby, animal health and welfare despite high performance? What do we need to know about modern farm animal physiology? Are specific behavioral patterns signaling animal welfare? Can anatomical, physiological and behavioral features be used to adjust breeding goals? What management and feeding regimen should be adapted to the needs of high-performing farm animals? The time span created by this abstract will be used to summarize and discuss the findings of session 3.

Rumen-protected B vitamins improved growth potential of female dairy calves after weaning

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Title: Rumen-protected B vitamins improved growth potential of female dairy calves after weaning Authors:Robert Roszkos, Ursula Blaeser, Dominique Bouchut Abstract: The objective of this study was to evaluate the effects of feeding a blend of rumen-protected B vitamins (RPBV; thiamine, folic acid, biotin, pyridoxine, and pantothenic acid) compared to negative control on the growth potential and performance parameters of weaned dairy heifers. After weaning, calves were allocated into 8 consecutive batches of 16-24 calves in each and received either (experimental) or no RPBV (control) supplementation in alternating manner. All calves were fed a liquid diet until weaning and were gradually introduced to a calf starter and a dry TMR starting at 10 days of age. RPBV (3 g/calf/day) was included to the calf starter from the first day of the trial. This starter feeding phase continued for 2–3 weeks post-weaning, after which corn silage was incorporated into the TMR and remained in the ration until the end of the trial (approximately 3 months per batch). The trial lasted ~80 days. Body weights (BW) were recorded at the beginning and at the end of the trial, as well as data of culled animals. Calves within each batch were born within 2 weeks and the overall calf age at the beginning of the trial was 87.5 days (STD = 6.9 days). Initial BW varied similarly to initial calf age (115.5 kg, STD = 5.4kg). Average daily gain (ADG) was calculated individually by dividing total BW gain by the total number of feeding days. Data were analyzed using ANCOVA procedure (XLSTAT software, v2024.4) using batch (1, 2, 3, 4) as a fixed effect. Initial BWs and age were used as a covariate in the analysis of ADG. Calves receiving RPBV gained 56 ± 34.7 g/d more than control during the feeding period (ADG: 1,043 vs. 1,099 g/day; P = 0.01). The ADG of calves of the RPBV group was higher in every single batches. Within the control, 2 calves were culled (reasons were: bloat, coccidiosis) and 3 others gained less than 700 grams per day during feeding. No calves were culled in the RPBV group and ADG was over 700 grams for all animals. In conclusion, the addition of RPBV to diet of growing dairy female calves can improve their performance and health status during the stressful post-weaning period.

Genetic Variant Mapping Across Multi-Tissue Transcriptomes Reveals Genome-wide Regulatory Signatures in Bovine IVP and MOET Calves

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In cattle breeding programs, in vitro production (IVP) and multiple ovulation and embryo transfer (MOET) are widely applied, however these reproductive strategies relate to transcriptome-wide gene expression patterns, and the landscape of expressed genetic variants has not been systematically characterized. Understanding the genome-wide genetic regulation of gene expression across different tissues is essential for deciphering the genetic architecture of complex reproductive traits. Although calves derived from these systems differ in developmental competence, metabolic adaptation, and reproductive performance, genotype–expression relationships across key endocrine and metabolic organs have not been systematically characterized. Therefore, the main objective of this study was to investigate the genetic control of gene expression in Hypothalamic–Pituitary–Adrenal–Gonadal (HPAG) organs central to bovine reproduction and fertility. We hypothesized that IVP and MOET calves exhibit distinct coding and regulatory variant profiles across major reproductive tissues and that functional SNPs may partially explain transcriptional differences. To address this, we performed multi-tissue RNA-seq of Adrenal, Hypothalamus, Liver, Muscle, Pituitary, and Testicle from eight calves (IVP n=4; MOET n=4), generating 48 transcriptome libraries. Variants were called using GATK, identifying ~300,000 SNPs per sample per tissue. Stringent multi-parameter filtration removed low-quality and strand-biased variants, producing a high-confidence transcriptome-derived SNP dataset. Functional annotation with ANNOVAR classified variants into synonymous, nonsynonymous, splice-site, UTR, intronic, and regulatory categories, with nonsynonymous and splice-site variants prioritized as potential coding-impact changes. Cross-tissue analyses revealed conserved polymorphisms alongside organ-specific variation. Comparison of IVP and MOET groups identified shared and group-specific SNPs, indicating both common genetic architecture and reproductive strategy–associated divergence. Across all tissues, a total of 741 differentially expressed genes (DEGs) were identified between IVP and MOET groups. Across tissues, approximately 600,000 high-confidence transcriptome-derived variants were detected per tissue group, of these nearly 25,000 SNPs per tissue mapped to 741 identified DEGs, representing ~4% of the expressed variant pool and highlighting a focused subset of variants potentially contributing to transcriptional divergence. DEG-prioritized cis-eQTL analysis (within ± 500 kb of flanking region of each DEG) was implemented to investigate genotype–expression associations and to strengthen inference on functional regulatory variants. This study establishes a multi-tissue RNA-seq-derived SNP resource for bovine reproductive biology and identifies candidate functional regulatory variants orchestrating the physiological and metabolic differences between IVP and MOET calves.

Milk yield dynamics reflect health of dairy cows in early lactation

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Dairy cows experience most health problems during early lactation, and these disorders can be associated with milk losses. Fluctuations in milk yield have been proposed as a valuable tool for health monitoring of dairy cows. The aim of this study was to investigate the relations between health status during the first 56 DIM and two types of milk yield dynamics: 1) lactation curve characteristics and 2) dynamic indicators of milk yield derived from deviations from the lactation curve. Data on daily milk yield and disease treatments of four previous experiments were combined and analysed. Cows were categorised in three ways based on disease treatments until 56 DIM: 1) health status groups: no health problem (NHP), systemic or inflammatory health problem (SHP), other health problem (OHP); 2) the number of disease treatments per cow (0, 1, or ≥ 2); and 3) the presence or absence of disease treatments for specific diseases including: mastitis, endometritis, claw and leg disorders, vaginal discharge or cystic ovaries. Lactation curve characteristics were calculated using the 5-day rolling average of daily milk yield and included peak production, day of peak production, slope to peak, total milk yield until 56 DIM, 305-d milk production and persistency. Dynamic indicators of milk yield, calculated from daily deviations in milk yield during 10-56 DIM and 10-305 DIM, included log-transformed variance (LnVar), lag-1 autocorrelation (Rauto), skewness and mean negative residuals (MNR). Mixed models were used to assess associations between disease treatments and milk yield dynamics. Peak production and total milk yield until 56 DIM were lower in SHP cows compared with NHP or OHP cows. Among cows of parity ≥ 3, SHP cows reached peak production later and had a lower slope to peak yield than NHP and OHP cows. Persistency and 305-d production were not affected by health status. Cows with health problems (SHP and OHP) during the first 56 DIM had a higher LnVar than cows without treatment for health problems (NHP). More specifically, cows treated for endometritis or cystic ovaries had a higher Rauto through lactation compared with healthy cows. The MNR during 10-56 DIM was lower for cows treated for claw and leg disorders compared with healthy cows, but MNR was not of added value for other treatments types or health groups, and neither was skewness. LnVar during 10-56 DIM was higher with increasing number of disease treatments. These findings indicate that cows with health problems in early lactation experience more milk yield perturbations than healthy cows, with responses depending on the type of disease and number of disease treatments in early lactation. In conclusion, while lactation curve characteristics specifically indicated cows with a systemic or inflammatory health problem, dynamic indicators of milk yield indicated both cows with a systemic or inflammatory health problem and cows with other health problems. Further research on disease-specific effects on milk yield dynamics may contribute to the development of on-farm health monitoring tools.

Liver hemorrhages and keel bone fractures in experimental cohorts of two high-performing laying hen strains from week 30 to week 42 of age

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Background Slaughterhouse studies revealed that liver hemorrhages and keel bone lesions commonly occur in high performing laying hens. Several risk factors associated with feeding, housing and production level have been identified. The present study aimed to describe the occurrence and the severity of these lesions in experimental cohorts of laying hens under standardized rearing, housing and feeding conditions. Methods The study comprised 438 hens (Lohman Selected Leghorn (LSL) n = 220; Lohmann Brown Classic (LB) n = 218) that had been reared under identical management, feeding and housing conditions in a free run indoor barn. Animals were slaughtered in four cohorts of 110 animals at 30, 34, 38 and 42 weeks of life. Three weeks before slaughter hens of the respective cohort were moved individually to metabolic units, equipped with a wooden perch, a nest, a feeding trough, water cups, and a wire mesh floor. In each cohort, 100 animals (LB n = 50; LSL n = 50) were fed a diet without mineral P supplementation (P_-) whilst ten animals, fed a diet with 1g/kg of mineral P (LB n = 5; LSL n = 5) served as controls (P_+). After opening, the abdominal cavity was inspected for bleeding before the liver was removed. Livers were photographed and the bleeding score, considering size and number of lesions, was assessed. A subset (P_-: LB n = 20, LSL n = 20; P_+: LB n = 5, LSL n = 5) was examined for keel bone fractures. Keel bones with adherent breast muscles were dissected from the carcass and examined by X ray. Fractures were scored using a visual analogous score. After maceration, keel bones were ashed. Data were analyzed in a three-factorial analysis of variance including strain, diet and age. Results Out of 438 animals, 9% showed no bleeding lesion in the liver, 66% had a score of 1 (< 10 lesions > 0.8 cm) and 25% (20% LB; 5% LSL) had a bleeding score of 2 (> 10 lesions > 0.8 cm) or higher. Strain exerted a significant effect leading to higher scores in LB than in LSL hens. Concerning keel bone fractures, 28% of the examined animals displayed a fracture score of 0, 36% a score of 1 and 36% a score of 2 and higher up to 4 (4%). Fracture severity was significantly higher in week 42 than in week 30. Omission of mineral P led to higher scores in LB but not in LSL hens. Cohort and diet significantly affected ash content. Ash content of keel bones was lowest in week 34 and highest in week 38. Controls consistently showed higher ash content than animals without mineral P supply. Ash content was negatively correlated with fracture score ($\rho = -0.27$) Conclusions The present study confirms the high prevalences of liver hemorrhages and keel bone fractures that are currently reported in commercial laying hens for the experimental cohorts studied herein, too. Strain exerted a significant effect on both scores, but lesions occurred in both strains. Thus, differences rather concerned severity than general occurrence of these lesions. Regarding fracture scores it must be considered that callus formation and mineralization occur two to six weeks after the preceding insult, implying that fractures might have been already present, when P_- diet was applied. Additionally, increasing scores over time might point to recurrent insults over time cumulating in week 42. Low correlation between ash content and keel bone fracture score further implies pathogenic mechanisms beyond bone mineralization.

Different motility of frozen semen between Holstein and Jersey bulls under heat stressed condition

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The bull fertility was known to be affected by hot humid conditions that is caused by global temperature increase. The increased body's temperature of cow's internal reproductive organs could reduce the fertility of bull's semen. It has been reported that fertility of Jersey is higher than Holstein in summer time primarily due to better tolerance of heat stress. However, the longevity of spermatozoa under heat stress has been less studied. To test tolerance of dairy bulls spermatozoa under unfavourable conditions, 4~5 proven bull semen of each breed were tested motility with time course examination. The spematozoa with progressive motility were isolated by Percoll gradient and exposured to 37.5°C and 41.5°C in a humidifed incubator with 5% CO2. The sperm was evaluated after 0, 1, 3 and 6 hours of incubation. We have found that longevity of sperm of Jersey frozen bull is better than those of Holstein. A significant increase ($P<0.05$) in Jersey spermatozoa motility was detected at 1 hour in 41.5°C and 3 hours in 37.5°C. These data suggests that sperm of Jersey breed have tolerance to heat stress during fertilization.

Phenotypic characterization of milk-derived liver enzymes and their associations with production traits in Holstein cows

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Milk biochemical markers provide a non-invasive approach for monitoring metabolic and liver status in dairy cattle. Understanding their variability across farms and lactation stages is critical for identifying phenotypes suitable for predictive models and genome-wide association studies (GWAS) targeting genetic determinants of metabolic efficiency and production traits. This study quantified milk-derived liver enzyme activities and their relationships with production and lactation parameters in 648 Holstein cows from three commercial Croatian farms (farm 1 – n = 344; farm 2 – n = 179; farm 3 – n = 125). Activities of alanine aminotransferase (ALT), alkaline phosphatase (ALP), and gamma-glutamyl transferase (GGT) were measured from milk together with standard milk composition traits, days in lactation (DIM), parity, and daily milk yield. Due to highly skewed distributions, ALT and ALP activities were log-transformed prior to statistical analysis. Descriptive statistics, Pearson correlations, and linear regression models were used to evaluate associations among traits. Substantial variability in milk enzyme activities was observed, particularly for ALT and ALP. ALT concentrations ranged from 0.6 to 1583.8 U/L, while ALP values ranged from 12.5 to 4198.5 U/L. GGT ranged from 223 to 12997 U/L. High coefficients of variation indicated considerable biological heterogeneity among samples. Correlation analysis revealed a strong positive association between ALP and GGT ($r = 0.59$), while ALT showed only weak correlations with the other enzymes. Days in lactation was moderately to high correlated with ALP ($r = 0.60$), indicating increasing ALP activity during later lactation stages. Negative correlations were observed between milk yield and ALP ($r = -0.40$) and GGT ($r = -0.25$), indicating potential links between metabolic load and production performance. Regression models further confirmed significant effects ($p<0.001$) of farm, days in lactation, parity, and milk yield on variation in milk enzyme activities,with ALP displaying the strongest associations ($R^2 = 0.47$). Overall, the results indicate substantial variation in liver enzyme activity among farms and across lactation stages, with ALP showing the strongest relationship with physiological and production variables. These findings suggest that milk-derived liver enzyme activities are associated with lactation dynamics and production traits, highlighting their potential as easily accessible phenotypes for metabolic monitoring in dairy cattle. The generated dataset provides a valuable phenotypic resource for future genome-wide association studies aimed at identifying genetic determinants of milk-based metabolic biomarkers.

The effect of half dose of colostrum replacer on immunity, feed intake, nutrient digestibility, and performance of dairy calves

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The aim of the study was to evaluate the efficacy of half dose of commercial colostrum replacer, as replacement of maternal colostrum, in newborn calves under different management conditions. The study has been conducted in two dairy farms where the calves were housed in individual pen with hutches with pelleted straw as bedding (farm1) or in individual cages with straw bedding (farm2). Furthermore, in farm1 calves were fed milk replacer by nipple buckets, whereas, in farm2, calves were feed milk replacer through individual automatic feeders. In both farms, the calves, blocked by birth weight, were immediately removed from the dams after birth and randomly allocated to one of the two experimental groups which received, within 3h from birth, either maternal colostrum (4l with ≥25°Brix via stomach tube, MC; n =30 in farm1; n =15 in farm2) or half dose of colostrum replacer providing 50g of IgG (4l via stomach tube, CR; n =30 farm1; n =15 farm2). In farm1, all the calves received a second meal of transition milk within 12h from the first meal. In farm2, only MC calves received a second meal of transition milk. The colostrum was analyzed for total bacterial count and E.coli. Blood samples were collected at birth and at 48h of life to estimate Apparent IgG efficiency of absorption (AEA). Following, blood samples were collected at 7 days of life and weekly thereafter until day 49. Daily, intake was recorded and calves monitored. Weekly, from birth to weaning (62±8 days), clinical evaluation, body weight, fecal score and bovine respiratory syndrome (BRD) score were recorded. Fecal and feed samples were collected every two weeks to assess nutrient digestibility. Average daily gain and nutrient digestibility were analyzed with ANOVA using the GLIMIX procedure; AEAI gG was analyzed, in Farm2 calves, by ANOVA using PROCGLM; all the other data were analyzed with ANOVA for repeated measures using the PROCMIX procedure. As expected, calves fed MC resulted in higher serum IgG levels compared to the CR fed calves ($P \leq 0.01$) at 48h of life. Furthermore, AEA IgG, measured in Farm2, resulted to be significantly lower in the CR group compared to the MC group ($P \leq 0.05$). However, only a trend for lower serum IgG levels was observed in the CR group over time with calves showing similar performance and health status. Furthermore, intake and nutrient digestibility didn't differ between groups. However, growth and performance were not affected. In conclusion, half dose of CR may be considered a valid replacement of maternal colostrum.

Association between passive immunity status on disease incidence and therapeutic interventions in dairy calves

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Transfer of passive immunity (TPI), achieved through colostrum intake and immunoglobulin (Ig) absorption, is critical for reducing morbidity and minimizing subsequent medication use in dairy calves. This study evaluated the correlation between serum IgG concentrations after birth and the occurrence of therapeutic interventions and disease incidence in dairy calves. This dataset included 1180 Holstein male calves, collected from dairy farms between 24-96 h after birth. Guidelines for colostrum management at the farm of origin included 3 feedings of high-quality colostrum (>22% Brix) within the first 24h, starting with 3-4L 1h after birth, followed by 2 feedings of 2L. At arrival at the research facility, serum IgG was measured within 24-96h after birth and categorized as: Poor (< 10 g/L), Fair (10.0 – 17.9 g/L), Good (18.0 – 24.9 g/L) and Excellent (≥25.0 g/L). Age at sampling was not included as covariates in the statistical model. General calf health was monitored daily. Diarrhea was defined as the presence of watery feces and visible clinical signs such as a dirty tail and dehydration. Diarrheic calves were treated with oral rehydration solutions and/or intravenous fluids. In severe cases, flunixin and/or trimethoprim-sulfadoxine were administered. A respiratory disorder was defined as a respiratory rate > 30 breaths per min, fever, and the presence of at least one additional clinical sign, such as coughing, nasal/eye discharge, or reddened mucosa, treated with oxytetracycline, florfenicol or tilmicosin in combination with meloxicam. Finally, cases of ear or navel infection, diphtheria and arthritis were treated with ampicillin trihydrate. Regardless of the underlying cause, fever was defined as a rectal temperature > 39.3 °C and treated with meloxicam. Time-to-treatment analyses were conducted using Cox proportional hazards regression (PROC PHREG, SAS). Multivariate logistic regression models (PROC LOGISTIC) were used to evaluate binary outcomes, risk factors, and disease cascade effects. Continuous variables were analysed using mixed-effects models (PROC MIXED). Serum IgG category was used as fixed effect. Compared to the Poor (n=96) group, Fair (n=490), Good (n=369), and Excellent (n=225) TPI reduced the hazard of respiratory disease (HR=0.62, 95% CI: 0.43-0.89, P=0.01; HR=0.53, 95% CI: 0.36-0.79, P<0.01; and HR=0.37, 95% CI: 0.24-0.58, P<0.01), fever (HR=0.55, 95% CI: 0.40-0.76, P<0.01; HR=0.48, 95% CI: 0.34-0.68, P<0.01; and HR=0.44, 95% CI: 0.30-0.64, P<0.01), and antibiotic usage (HR=0.59, 95% CI: 0.43-0.81, P<0.01; HR=0.47, 95% CI: 0.34-0.66, P<0.01; and HR=0.38, 95% CI: 0.26-0.55, P<0.01). Overall morbidity was significantly lower in the Good (HR=0.69, 95% CI:0.51-0.94, P=0.02) and Excellent (HR=0.63, 95% CI:0.45-0.87, P<0.01) groups, whereas the Fair group only tended to have a reduction (HR=0.77, 95% CI:0.58-1.03, P=0.09) compared with Poor. Immunity status did not affect the hazard of receiving a treatment for diarrhea. However, calves previously treated for diarrhea had 2.28 times higher odds of developing a respiratory disease (95% CI: 1.71-3.04; P < 0.01). Calves in the Excellent group (2.61 d) had fewer treatment days compared to calves in Fair (3.19 d) and Poor groups (3.35 d; P=0.06). Serum IgG concentrations above 18.0 g/L were associated with a lower risk of overall morbidity in dairy calves, reduced incidence of respiratory disease and decreased need for antimicrobial treatments. Dairy farming practices should aim to achieve higher IgG concentrations, specifically ensuring calves fall within the Good classification, as a minimum management goal, while striving for Excellent.

Effect of incorporation a spray-dried fat concentrate containing tributyrin and tricaproin into milk replacer on the need for medical interventions in newborn calves.

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The presence of butyric (C4:0) and caproic acids (C6:0) in milk replacers (MR) is minimal or absent compared to bovine milk. The objective of this study was to evaluate the effect of inclusion of a spray-dried fat concentrate containing tributyrin and tricaproin as sources of C4:0 and C6:0 in MR on the need for medical interventions in newborn calves. This study analyzed data from 4 experiments conducted at the same facility, involving 138 Holstein male calves (2.2 ± 0.10 d of age; 45.5 ± 0.53 kg BW; 18.8 ± 0.72 g/L of serum IgG) collected from dairy farms. Calves were blocked by arrival sequence and within each block, calves were randomly assigned to either control MR (CON, n=69) or a MR containing tributyrin and tricaproin (TBTC, n=69). Milk replacers were iso-energetic with 23.8% protein, 27.5% fat, and 35.1% lactose (as product basis) and fed upon arrival. In 2 out of 4 experiments calves were group housed, fed MR ad libitum and monitored until d 84 and d 91. In the last 2 experiments calves were individually housed and fed MR twice daily until d 21 or d 35. Use of oral rehydration solution (ORS) was investigated separately from total medical treatments as it is considered a non-invasive supportive intervention. Medical interventions were analyzed using Cox proportional hazards regression (PROC PHREG in SAS) to estimate hazard ratios (HR) with 95% confidence intervals (CI). Continuous variables were analyzed using mixed-effects models in PROC MIXED. Treatment and block nested within experiment were included as fixed effects in both analyses to account for experimental heterogeneity while estimating a pooled treatment effect across experiments. Discrete variables were evaluated using a binomial logistic regression model (PROC GENMOD). The proportion of calves removed from the study due to mortality or severe health issues did not differ between TBTC and CON (2/69 vs. 4/69; P = 0.40). Calves fed TBTC had a 74% lower hazard of requiring any medical intervention compared with CON (HR = 0.26, 95% CI: 0.13-0.53; P < 0.01). Similarly, calves fed TBTC had a 68% lower hazard of receiving an antipyretic medication (HR = 0.32, 95% CI: 0.15-0.66; P < 0.01), a markedly lower hazard of receiving medical treatment for diarrhea, excluding ORS (1/69 vs. 8/69; HR = 0.01, 95% CI: 0.0004-0.32; P < 0.01), and a 88% lower hazard of antibiotic use compared to CON (HR = 0.12, 95% CI: 0.05-0.28; P < 0.01). Calves fed TBTC had 82% lower hazard of receiving at least one intervention for respiratory disorders (HR = 0.18, 95% CI: 0.08-0.42; P < 0.01) and 96% lower hazard of a second treatment for respiratory disorders than CON (2/69 vs. 10/69; HR = 0.04, 95% CI: 0.01-0.34; P < 0.01). No differences were observed in ORS use, either in the number of calves requiring ORS (P = 0.71), the hazard of ORS administration (HR = 1.01, 95% CI: 0.48-2.14; P = 0.98), or the number of ORS portions used per calf (P = 0.63). Calves fed TBTC required fewer days of medical treatment per calf than CON (0.63 vs 1.60 d; P = 0.01), including fewer days of intervention for respiratory disorders (0.21 vs 0.80 d; P < 0.01). Inclusion of tributyrin and tricaproin in MR reduced the incidence of health disorders and the need for medical interventions in newborn calves.

Digestive development and behavior in calves weaned on hay or concentrate diets

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Young animals are often fed high-starch diets to enhance growth performance and gastrointestinal tract (GIT) development. However, this likely shifts the ruminal pH below the physiological range, possibly affecting rumen development. We hypothesized that a high-starch diet in the initial weeks of life would significantly influence feed intake, behavior, growth performance, and digestive development. We randomly allocated 48 newborn male calves to 2 treatments and fed milk replacer with ad libitum access to either chopped hay (HAY; crude protein 18.2%, NDF 41.9% and stach 1.96% dry matter) or a high-starch diet (STARCH; CP 18.0%, NDF 23.1% and starch 40.3% DM) for 10 weeks. In the last week, feed intake, digestibility and retention time were measured on a subset (n = 7/treatment). Afterwards, these animals were slaughtered for gross measurements and sample collection. Animals were continuously video recorded during the entire experiment and one day in weeks 2, 7, 10 of the study was selected for detailed behavior analysis. Groups were compared using mixed models that accounted for individuals and calf batch for repeated measures, and only for batch as a random effect in single measures. Body mass and solid feed intake increased significantly over time but did not differ between groups (all calves). In the subset of selected calves, at similar dry matter intake, STARCH showed higher dry matter and protein digestibility ($P < 0.01$) but lower NDF intake and NDF digestibility ($P < 0.01$). STARCH had longer mean retention times for fluid ($P = 0.04$), small particle ($P < 0.01$) and large particle marker ($P < 0.01$), and greater mean particle size of feces ($P < 0.01$). HAY had greater proportions of fine particles (<0.25mm, $P < 0.01$). The proportion of the finest fraction was higher in HAY ($P < 0.01$). At slaughter, there was no body mass difference between the groups, but a heavier carcass weight in STARCH ($P = 0.04$). STARCH had higher tissue mass for the caecum, jejunum, and total small intestine ($P < 0.01$; $P = 0.04$; $P = 0.02$), but not different for rumen. Although the total short-chain fatty acid amount did not differ, the rumen propionate and butyrate proportions in STARCH were higher ($P < 0.01$, $P = 0.02$) at the expenses of acetate. The pH of rumen, abomasum and caecum contents were higher in HAY ($P < 0.01$; $P = 0.04$ and $P < 0.01$). The spleens of STARCH were larger ($P < 0.01$). For behavior(all calves), animals in the HAY group spent more time on eating and ruminating, with a corresponding reduction in resting time. Conversely, the STARCH group exhibited increased stereotypic behavior and bedding consumption. These results indicate that early life high-starch diet need not be advantageous in terms of growth and GIT development if high-quality forage is available, and that high-starch diet has the expected effects on digestibility, rumen fermentation and behavior. Whether the low rumen pH in first weeks of life is detrimental for animal health, especially later in life, will be assessed in the older animals from this experiment. The absolutely and relatively larger spleens of the STARCH group may suggest a higher immunological challenge, though confirmation with immune markers is needed. Funded as WEAVE program by the National Science Centre Poland (2021/43/I/NZ9/02222) and Swiss National Science Foundation (310030E_209573).

Effects of calf dry TMR and warm-air-dried hay as sole solid feed on growth and behavior of pre-weaned calves

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Solid feed intake is essential for early rumen development in calves. Calf dry total mixed rations (TMR), typically consisting of concentrate and chopped straw, are commonly used as the sole solid feed for unweaned dairy calves on German farms. Hay could represent an alternative feed source but typically contains less energy and protein. Warm-air-dried hay has high sugar content and improved digestibility and may represent an on-farm alternative to calf dry TMR. Furthermore, previous studies report increased rumination and reduced cross-sucking in calves offered hay.This study evaluated warm-air-dried hay as an alternative to calf dry TMR for feeding pre-weaned calves, focusing on growth, performance, and behavior.24 Fleckvieh calves were purchased at auction and transported to the experimental facilities of the Hofgut Neumühle on the same day. Calves had an average age of 32.1 ± 6.7 days and a body weight of 86.2 ± 3.2 kg. Calves were randomly assigned to two weight-balanced groups (n = 12) housed in identical barns. One group received warm-air-dried hay throughout the experimental period (HAY), while the second group received a conventional calf dry TMR (TMR-G), which was weighed and replaced daily. No other solid feed was provided.Calves received 10 L of milk replacer per day (140 g/L) via an automatic feeding station for four weeks. From week four onward, milk allowance was gradually reduced over four weeks until calves were weaned in week eight, followed by two weeks of monitoring. Growth was assessed weekly by body weight and body measurements (e.g., body length). Calf behavior was recorded, capturing 8 hours (08:00–16:00) on two days per week. Behavior was analyzed using BORIS Software and an ethogram comprising 15 behaviors. Three focal animals per treatment were selected for detailed behavioral analysis in addition to scan sampling of both groups. Animal behavior, development data, and feed intake were analyzed using separate generalized linear models.Calves fed TMR consumed more solid feed dry matter (1.1 kg/d) compared with calves fed hay (0.8 kg/d). Accordingly, the intake of metabolizable energy and utilizable crude protein (nXP) was higher in TMR-G than in HAY (12.9 vs. 9.01 MJ ME; 191 g vs. 124 g), with significant differences observed during the final two weeks of the trial. Milk replacer intake differed only on day 20 of the trial, with higher intake in HAY compared to TMR-G. Body weight was numerically higher in TMR-G during the final two weeks but did not differ significantly between treatments. Body measurements only differed in greater body length in TMR-G on day 4 of the trial. Behavioral analyses showed that calves in HAY spent significantly more time feeding and ruminating compared to calves in TMR-G. In contrast, calves in TMR-G spent significantly more time lying and exploring their environment. Although tongue rolling was only rarely observed, it occurred significantly more often in TMR-G than in HAY.Overall, the results indicate that warm-air-dried hay can serve as an adequate sole solid feed during the early weeks of the rearing period but does not sufficiently meet energy and protein requirements during the weaning phase, when nutritional dependence on solid feed increases. Furthermore, the type of solid feed influenced calf behavior, with mixed effects regarding the relative advantages of hay and calf dry TMR.

Factors influencing voluntary free water intake of Holstein calves with high milk replacer allowance and slow, late weaning

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The current notion is that free water intake reflects a physiological integration of osmotic load, rumen activity, thermoregulation, and milk dilution effects, rather than being solely triggered by weaning onset. The aim of this study was to retrospectively analyze the water expense (WE; water use in L after automatic detection of a calf in the water station, WS) of 30 Holstein-Friesian calves (20 female, 10 male) fed a high milk allowance until d 56 and then gradually weaned until d 98. Calves were reared in three seasonal batches, with weaning between November to January (n=11), March to May (n=10), and April to June (n=9). All calves received 4 L colostrum and 12 L/d of MR until weaning. Concentrate (CON), hay, and water were offered ad libitum. Water temperature was < 10°C during the first weaning period and > 20°C during the second and third. Data were analyzed using R-Studio with a linear mixed model, including pattern and age as fixed effects and seasonal batch as a cofactor. Batch effects were tested with the partR2package by comparing the partial R^2 of batch and pattern, as well as by including their interaction in the model. First WE was defined as the first WE in the WS exceeding 0.1 L for at least two consecutive days. To examine its relation to the weaning process, calves were assigned to three behavioral patterns based on when first WE occurred relative to the weaning timeframe. In 5 calves, first WE was recorded before the onset of weaning (”BEF”, d 28 to d 56); in 15 calves, during the onset of weaning (“DUR”, d 59 to d 77); and in 10 calves at the end of weaning (“LAT”, d 81 to d 93). BEF calves may exhibit increased exploratory behavior, indicated by visits to the WS at a younger age (wk 4 ± 0.04 in BEF, wk 5 ± 0.07 in DUR and LAT, P = 0.007). Age at first WE coincided with overall number of visits to the WS/d (P < 0.001) as well as visits with WE per day (P < 0.001), with the highest values in BEF and the lowest in LAT calves. An earlier WE corresponded with a higher WE per day and per visit (P < 0.001). MR intake did not differ (P = 0.214), while LAT calves were associated with higher CON intake (P < 0.001). Feeding behavior did not differ among calves. The three seasonal batches were a significant cofactor in all models. The variance explained by batches was greater than by behavioral patterns (partial R^2= 0.09 and partial R^2= 0.01, respectively), in addition to an interaction between batch and pattern (P < 0.001), suggesting a batch effect due to the different seasonal batches. The winter batch had the first WE at the highest age and the lowest WE, per day (P = 0.002) and per visit (P = 0.008). The CON intake in the winter batch was higher than in the other two (P = 0.002). No behavioral pattern in feed intake can be linked to the onset of free water intake, and the behavior seems to be highly individual in well fed calves. Continuous access to drinking water is necessary even with high milk allowance, as water intake is not uniformly synchronized with weaning but reflects individual developmental paths.

Motivation of dairy cows to access food diversity in an operant conditioning task

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When given the opportunity, dairy cows choose to eat a diverse diet. However, in conventional systems, they are mainly fed with total mixed rations (TMR). This kind of diet may not meet their behavioural needs since it is poorly diversified, monotonous and do not allow them to perform their natural behaviour of feeding choices. In order to determine the importance of food diversity from the dairy cows' perspective, this study aimed to evaluate the motivation of 24 dairy cows to access food diversity, depending on whether they have been accustomed to a diverse diet or not. To do so, half of the cows was submitted to a period of food diversity for 29 d: they were fed with their usual TMR and two hays of contrasted nutritional values (regrowth of fescue and 1st cut of permanent pasture) offered separately. The other half was fed exclusively with the TMR. All cows performed two sessions of an operant conditioning test (15 d before and 15 d after the food diversity period). During the 10-min test, they could choose to eat their usual TMR freely available or walk across the 16 m U-turn test area to access two different foods of contrasted nutritional values (beet pulp pellets and permanent pasture hay) offered in limited quantities each time they completed a turn. During the first session, the cows only walked 0.28 ± 0.09 turn, chose to eat a large amount of TMR (4.0 ± 0.2 kg) and few diversified foods (no beet pulp and 15 ± 3.6 g of hay), showing that they preferred the TMR and were not motivated to access the diversified foods. During the second session, whether they had been accustomed to a diverse diet or not, the cows also chose not to provide the effort to walk to access the diversified foods. They walked 0.23 ± 0.08 turn and ate 3.9 ± 0.2 kg of TMR, no beet pulp and 13 ± 4.1 g of hay. Our results suggest that, under our experimental conditions, the effort required to access the diversified foods was probably too high in relation to the benefits derived from these foods, which could have been considered insufficiently rewarding. Moreover, habituation to a diverse diet did not affect the cows' motivation to access the diversified foods. These results would need to be confirmed under other experimental conditions (with other food rewards and/or effort of lower intensity).

Enhancing mineral bioavailability, stress resilience and welfare using magnesium phosphate: evidence from in vitro models and swine in vivo trials

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Context.Magnesium (Mg) and Phosphorus (P) availability is essential in pig nutrition to support health, performance, and to reduce excretion. This study evaluated a magnesium phosphate source (MgP) in comparison with monocalcium phosphate combined with magnesium oxide (MCP+MgO), through in vitro and in vivo approaches. In vitro work assessed mineral solubilization, absorption, cell viability under stress, and modulation of key molecular pathways. In vivo trials examined MgP effects on tail biting, ear lesions, and scratches in piglets. Materials and Methods. In vitro, MgP was compared with an iso-P and iso-Mg inclusion of MCP+MgO. P and Mg solubilization were quantified using an in vitro digestion model, and absorption assessed with a Caco-2 uptake assay. Cell viability was evaluated under normal conditions and under heat (40 °C) or oxidative stress (1000 µM H_2O_2). Gene expression related to Mg transport, membrane integrity, and oxidative stress responses was quantified by qPCR. A minimum of four replicates was used per assay. Data were analyzed by ANOVA ($p<0.05$). In the first in vivo trial, 398 piglets (70 days old) were assigned to either a control diet (MCP+MgO) or an MgP diet (iso P, Mg, Ca). Tail lesions were scored from 0 to 3 on D0, D18, D33, D42, D67, and D81. Weights were recorded at D0, D42, and D81. Weight data were analyzed by ANOVA and lesion scores by Mann-Whitney test ($p<0.05$). In the second in vivo trial, 151 gestating sows, were assigned to either control (MCP+MgO) or treated groups (MgP+MCP). After farrowing, piglets were allocated into two groups, each group having half of the piglets from first sow group and half from the second. Piglets' ear lesions and scratches were assessed at D15 post-weaning. Data were analyzed by ANOVA ($p<0.05$). Results.MgP significantly increased in vitro Mg solubilization and absorption compared with MCP+MgO ($p<0.01$). Under normal, oxidative or heat stress conditions, MgP increased cell viability compared with MCP+MgO-treated cells ($p<0.05$), and showed greater antioxidant capacity ($p<0.01$). Under normal conditions, MgP upregulated TRPM6, SLC41A3, and CLDN1, indicating improved Mg transport and membrane integrity ($p<0.05$). It also modulated oxidative pathways by maintaining baseline GPX2 expression, while MCP+MgO induced its upregulation ($p<0.05$). Under oxidative stress, MgP decreased CAT and increased GPX2 expression, suggesting a propensity toward chronic antioxidant responses rather than the acute detoxification ($p<0.05$). In the first in vivo trial, MgP reduced tail-lesion scores throughout the study ($p < 0.05$), with an average 20% decrease and a 66% reduction in severe lesions (score 3). Average daily gain was also improved between D0 and D42 (795 g/d vs 755 g/d) for MgP group. In the second in vivo trial, piglets from treated sows showed fewer ear lesions (4% vs 8.4%, $p<0.001$) and a tendency toward fewer scratches ($p=0.07$). Treated piglets also showed reduced ear lesions (4.9% vs 7.7%, $p=0.01$). The lowest lesion rate was observed when both sows and piglets were treated, with a 2.9-fold reduction ($p<0.001$). Conclusion.In in vivo trials, MgP reduced tail biting and ear lesions while supporting performance. This effect may be associated with increased Mg bioavailability, enhancing its metabolic utilization and supporting its role in stress-related physiological pathways. These results identify MgP as a promising alternative to MCP+MgO for improving mineral utilization, stress resilience, and welfare in pig.

Influence of dietary tryptophan-to-lysine ratio and level of large neutral amino acids on performance, lesion scores and behaviours of weaned piglets

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Tryptophan (Trp) competes with other large neutral amino acids (LNAA) for transport across the blood–brain barrier. This can influence its availability in the brain for serotonin synthesis, thereby affecting pig behaviour and indirectly influencing growth through changes in appetite and stress regulation. We aimed to study the effect of dietary Trp level on behaviour and biting-related damage in weaned piglets fed diets with adequate or reduced LNAA levels. Post-weaning piglets (n=720) with intact tails were assigned to one of 10 dietary treatments in a 5×2 factorial design, varying in standardized ileal digestible (SID) Trp:Lys ratio (0.16, 0.20, 0.24, 0.28, and 0.32) and LNAA level (85% or 100% of CVB requirements), with 12 pens (6 piglets, balanced by sex) per treatment. Piglets were classified into body weight (BW) classes (light, medium, and heavy). Feeding the LNAA 100% diet increased average daily gain (ADG) and gain-to-feed ratio (G:F) compared to the LNAA 85% diet across all BW classes ($P<0.01$). A Trp x BW class interaction was found for G:F ($P<0.05$). Within BW classes, dietary Trp affected G:F in heavy piglets, whereas no effect was detected in light or medium piglets. The response of G:F to dietary Trp in heavy piglets was nonlinear, with the highest G:F was found at Trp 0.28, values being higher than for Trp 0.20 and 0.32. Percentage of time spent walking, standing, and sitting was lower in heavy than in light piglets ($P<0.05$). Piglets fed the LNAA 85% were more active than those fed the LNAA 100% diet ($P<0.05$). Lying-inactive behaviour was higher in piglets fed Trp 0.24 than in those fed Trp 0.16 ($P<0.05$) and was greatest in heavy piglets ($P<0.01$). Tail, ear, and skin damage increased over time ($P<0.01$). Additionally, a Trp×LNAA×BW class interaction was observed ($P<0.05$) for both tail damage severity and skin lesion score; however, no pairwise differences were detected after Tukey adjustment. Heavy piglets exhibited the highest skin lesion severity ($P<0.01$), while ear damage was lower in heavy than in medium piglets ($P<0.05$). Neither dietary Trp nor LNAA affected the frequency of tail and ear biting, although both behaviours increased from week 3 to week 5 ($P<0.01$). Aggressive behaviour decreased over time ($P<0.05$) and was lower in piglets fed Trp 0.24 and 0.28 than in those fed Trp 0.16 ($P<0.01$). Similar effects of Trp level were found for mounting at the feeder ($P<0.01$). Belly nosing was reduced in piglets fed Trp 0.20 and 0.24 compared to Trp 0.16 and 0.32 ($P<0.05$). Heavy piglets showed less ear biting and belly nosing than light and medium piglets ($P<0.05$). In conclusion, feeding weaned piglets with Trp levels relative to Lys of 0.24 – 0.28 improved feed efficiency in heavy piglets and increased lying behaviour while reducing aggression, belly nosing and feed competition behaviour, regardless of LNAA supply. The response to the Trp:Lys ratio was non-linear; both deficiency (0.16) and excess (0.32) produced more undesirable behavioural. Tail and skin damage were affected by a Trp×LNAA×BW class interaction, with no clear pattern. Moreover, adequate dietary LNAA improved growth performance and increased lying behaviour across all BW classes.

Comparison of two commercial 6-phytases on growth performance in a dose response trial with weaned Piglets

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Phytases are widely used in piglet nutrition to enhance the digestibility of phytate-bound phosphorus (P) and calcium (Ca), supporting growth while reducing inorganic P excretion. This study evaluated the dose–response efficacy of two commercially available 6-phytases on growth performance and apparent total tract digestibility (ATTD) of Ca and P in weaned piglets. A total of 384 piglets (Tempo × TN70; 29 days of age; 8.5 ± 1.05 kg BW) were assigned to eight treatments: a P-deficient negative control (NC), a P-adequate positive control (PC), and six NC-based diets supplemented with 250, 500, or 1000 FTU/kg of either product A or product B. Both products are registered with EFSA and are produced by genetically modified strains of Komagataella and Aspergillus oryzae for products A and B, respectively. Experimental diets in pelleted form were fed from day 8 to 35 post-weaning. Growth performance was monitored throughout the study, and pen faecal samples collected from days 22–25 were analysed for ATTD of P and Ca. Statistical analysis was conducted using GenStat® for windows (23rd edition). Across all performance parameters, no significant differences were observed between products at any of the inclusion levels. During days 8–22, product A at 500 FTU/kg (486 g/day) improved ($P<0.05$) ADG by 14.6% compared to NC (424 g/day). For the overall period (days 8–35), PC improved ($P<0.05$) the ADG and feed conversion ratio (FCR) compared to the NC (562 g/day vs. 483 g/day; 1.33 vs. 1.45 respectively). Product A and B at 500 and 1000 FTU/kg also improved ($P<0.05$) ADG compared to the NC (575 and 561 g/day for product A; 553 and 555 g/day for product B). The FCR of piglets fed Product A at both 500 and 1000 FTU/kg and product B at 1000 FTU/kg did not differ from the PC group. Both phytases improved ($P<0.05$) mineral digestibility in a dose-dependent manner. Further, the faecal score during the period days 22-35 improved ($P<0.05$) with increasing phytase doses. Dose–response modelling for ATTD of Ca and P demonstrated strong linear relationships for both phytases, supporting a consistent dose-response relationship (R^2= 62% to 91%, for Ca and P respectively). In conclusion, both 6-phytase products were effective in hydrolysing phytate-P and improve growth performance and mineral digestibility in a wheat-barley-soybean meal-based NC diet, particularly at the dose of and above 500 FTU /kg.

Effects of Early-Lay Prebiotic Supplementation with Galacto-oligosaccharides on Severe Feather Pecking and Associated Feather Damage in Laying Hens

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During severe feather pecking (SFP), one hen repeatedly pecks or pulls the feathers of another, leading to feather damage (FD), increased risk of injury and pain, and impaired thermoregulation and locomotion. These effects compromise welfare, health, and productivity. Potent stressors, like the loss of social and environmental familiarity, can promote frustration and SFP. This can occur during the transition from rearing to laying barns, which involves transport, remixing of groups, and adaptation to new pens. In birds, preening and pecking can also socially transmit chemicals and microbes via feathers, potentially shaping social interactions. Disturbances of the microbiota-gut-brain axis have also been linked to SFP. Prebiotics, like galacto-oligosaccharides (GOS), can increase the growth of beneficial bacterial taxa in the gut and could serve as preventative tools to enhance birds' capacity to cope with subsequent stressors. This study tested if preventative GOS could reduce SFP and FD during subsequent periods of social mixing and adaptation to new pens. White Leghorn laying hens (N=144) from genetic lines selected for high or low FP activity were housed in 12 floor pens with equal line representation and observed for FP and FD during a baseline period (18-19 weeks of age; woa). During the supplementation period (19-22 woa), six pens (GOS) received water supplemented with Nutrabiotic® GOS (74% GOS; Dairy Crest Limited, Davidstow, United Kingdom), and six received plain water (control). This was followed by a stress period (23-26 woa) where half the GOS and control pens underwent repeated mixing of unfamiliar hens and were moved to new pens (stressed), while the others remained undisturbed (undisturbed). SFP was analyzed from videos as average pecks per pen. FD was scored (0-2) by a blinded observer at the end of each period. A generalized linear mixed model (SAS) with a lognormal distribution was used to evaluate the effects of supplementation and stress on SFP during each period. Behaviour during the baseline period served as a covariate and pen was fitted as a random effect. FD was analyzed with multinomial models reporting odds ratios (OR and 95% confidence interval (CI)) for worse damage. Preventative GOS supplementation did not change SFP rates during supplementation ($p = 0.3935$), or the subsequent stress period ($p = 0.2783$). However, GOS hens were more likely to have worse FD during the supplementation period than control hens (OR=2.20, 1.08-4.49). During the stress period, both undisturbed GOS hens (OR=3.96, 1.47-10.70) and stressed GOS hens (OR=2.92, 1.08-7.89) were significantly more likely to have worse feather cover damage than undisturbed control hens. Based on these results, we propose two explanations: 1) a sensory/social transmission pathway, where GOS and its galactose-containing residues on beaks and feathers reinforce early pecking and 2) a reward-loss frustration pathway where withdrawal of the preferred GOS at stress onset redirects pecking. This interpretation aligns with evidence that hens can detect galactose and exhibit preference-driven intake responses to it, and highlights new opportunities for hypothesis-driven work on feeding strategies to optimise welfare.

Location Matters: Impact of Pecking Block Placement on Feather Condition in Laying Hens

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Feather pecking (FP) in chickens is the act of pecking and/or pulling at the feathers of conspecifics, often leading to feather damage (FD) and reduced welfare. FP is widely believed to be a form of redirected foraging behaviour as under natural conditions, chickens forage on the ground and peck upwards to consume berries from bushes. However, in commercial environments chickens are housed at high stocking densities and food is generally provided at ground level, increasing the likelihood that upward pecking behaviour is redirected towards the feathers of other birds. Introducing elevated pecking blocks (PB) as edible enrichment has the potential to redirect FP away from conspecifics and towards the blocks. This study aims to investigate whether elevating PB could redirect attention upwards and influence the onset and progression of FP behaviour, feather cover scores, and PB usage. A total of 364 1-day-old chicks from two commercial strains were assigned to one of three treatments: access to hanging PB, ground PB, or no PB (n=4 pens/treatment). Feather cover damage was scored by blinded observers across four regions (head/neck, back, tail, and belly) and the worst feather score per bird (0=no damage, 1=minimal damage, 2=severe damage) was used for statistical analysis. A multinomial model with odds ratios (SAS V9.5) was used to analyze the effect of treatment on FD where an OR > 1 indicates higher odds of worse FD. All birds exhibited intact feather cover at 17 weeks of age (WOA). At 30 WOA, 73.1% of birds retained intact feather cover, while 18.6% and 8.3% showed minimal and severe FD, respectively. At 45 WOA, feather condition declined across treatments, with 41.8% of hens maintaining intact feather cover with the rest of the birds displaying minimal (30.8%) and severe (27.4%) FD. Multinominal modeling assessing the odds of receiving the worst FD score indicated no differences between control and ground PB treatments, at any age. At 45 WOA, hens provided with ground PB had significantly greater odds of receiving the worst feather damage score compared to no PB (OR = 1.69, 95% CI: 1.06–2.70, p = 0.027), indicating a higher likelihood of severe feather damage in the ground PB treatment. In summary, feather cover declined with age across all treatments, highlighting a general welfare concern; however, differences among enrichment placements were minimal under the conditions of this study. Ongoing analyses will evaluate whether individual pecking block usage and behavioural observations, such as FP behaviour, correspond with FD outcomes and are influenced by the placement of PB, with findings interpreted in the context of scoring methodology, environmental factors, and the challenges of disentangling causes of feather loss in enriched research settings.

Impact of dietary amino acid supply on milk urea and urine nitrogen excretion and energy corrected milk of dairy cows fed hay-based diets

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This study aims to investigate the impact of amino acids supply from hay-based diets on lactating dairy cows (LC) N excretion and energy corrected milk ECM (lNEx or hNEx). Twenty herds (184±89 cows; 178±19 DIM; 678±33 BW) were enrolled in the study. Feed intake was measured in each farm during 2 consecutive days by difference between the TMR offered and refused. Urine samples were collected twice from 10% of the LC. Milk yield was recorded, and 2 bulk milk samples were collected at 12h intervals. The BW was measured on 20% of the LC. The urinary creatinine content was determined through the Jaffe colorimetric method and used to calculate urinary output. Urine nitrogen (UN) and Milk Urea Nitrogen (MUN) were determined by Dumas and Milkoscan respectively and summed for NEx calculation. The dietary aminoacid (AA) content was estimated using the internal standard method (Trp), and an external calibration curve (all other AA) after analysis of the samples through RP-UPLC/ESI-MS in the SIR scan mode. The herds were categorized as low or high NI (<600, 601 to 650 and >650 g/d respectively) and, within each intake category, as lNEx or hNEx (respectively the lower and higher 50% of the data). The NEx was reduced by the increase of dietary serine (y = -100.29x +530.46; R_2=0.155), threonine (y = -113.63x+529.88; R_2=0.148) and tyrosine (y = -70.137x+360.95; R_2=0.127), while it was increased by the increment of dietary lysine (y = 66.179x+104.15, R_2=0.178). The ECM was increased when dietary arginine increased from 2.5 to 4.0 g/MCal (y=2.0579x+26.819; R_2=0.130). The lNEx herds received higher content of Met (0.26±0.03 vs 0.24±0.02% DM; P=0.040) and Ser (0.66±0.07 vs 0.61±0.04% DM; P=0.034), and a trend for a higher content of Ala (0.067±0.04 vs 0.64±0.05% DM; P=0.058), Thr (0.57±0.06 vs 0.54±0.03% DM; P=0.090), and total AA (12.08±0.87 vs 11.40±1.01% DM; P=0.061). These differences disappeared when the amount of amino acids administered were expressed on an energy base (g/Mcal). The NEx from LC fed hay-based diets seems to be reduced by the administration of specific amino acids and the role of dietary energy should be considered in modulating NEx. Research supported by the European Union's H2020 research and innovation program, G.A. No 777974.

Whole plant vetch silage as roughage for growing pigs: Effects on growth performance, slaughter characteristics and outdoor run use

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Roughage production can contribute to soil and crop health in circular agricultural systems, and improve the resilience of systems with pig husbandry by providing opportunities to conduct natural behavior, improving health, and contributing to the nutrient supply (reviewed by Klevenhusen et al. 2026). Vetch plants can be included in crop rotations as winter catch crops, effectively supplying nitrogen for the following crop, while providing a protein-rich roughage. The objective of the study was, to investigate if vetch whole plant silage can contribute to the nutrient-supply of growing pigs and enrich their husbandry conditions. In two trials (DEZ and MAY), 60 and 80 growing pigs ((Topigs Norsvin L-Line x Topigs Norsvin Z-Line) x PIC 408) born and raised on the experimental station of the Thünen Institute (certified according to EU regulation 2018/848), were fed a compound feed containing cereals and grain legumes harvested on the station. In addition to the compound feed for the growing and finishing phase, formulated to contain about 8.2 and 5.7 g standardized ileal digestible lysine and less than 176 and 134 g crude protein per kg dry matter, respectively, 20 pigs each per trial were supplied either with triticale straw (control = CON) or with 0.9 and 1.4 kg fresh whole plant vetch silage derived from Pannonian vetch (VP), common vetch (VS) or hairy vetch (VV, only in MAY) in the growing (<75 kg body mass) and finishing phase, respectively. The pigs were weighed weekly, and slaughtered when weighing at least 119 kg. Dressing percentage was calculated using the warm carcass mass and lean meat percentage was measured using FOM S89 (SFK systems, Copenhagen, Denmark). Retention time of the silage in the racks (every day) as well as use of the outdoor run (last two Wednesdays and Saturdays before first slaughter) were observed for CON, VS and VP using photographs that were automatically taken every 10 minutes (Dahua HDW1200, 2 MP; Dahua XVR5116H-X). Generalized linear mixed models were performed with R. Pigs in the silage groups had a 3-5 d shorter growing and 3-5 d shorter finishing phase as well as an improved compound feed conversion in the finishing phase. Dressing percentage was between 79 and 80 % and lean meat percentage was 56.9 (CON), 57.7 (VS) and 58.2 (VP, VV). During daytime (DEZ 08:30–20:30; MAY 07:00–21:00) pigs of VP and VS were observed more often in the outdoor run and in front of the racks. While CON racks were never emptied, VP and VS groups failed to empty the racks on 6 and 7 days in MAY, respectively. On the other days, VP and VS groups emptied their racks during 3.2 ± 0.65 h in MAY and 6.3 ± 3.34 h in DEZ and 2.5 ± 0.65 h in MAY and 6.6 ± 3.34 h in DEZ, respectively. Like alfalfa silage (Messinger et al. 2022), vetch silage can contribute to the nutrient supply and improve compound feed efficiency without decreasing carcass quality. Outdoor run use increases with providing grass or clover silage (Høøk Presto et al. 2009, Holinger et al. 2018, Höne et al. 2022). Vetch silage has the same effect. Overall, vetch whole plant silage is a suitable roughage for fattening pigs that can contribute to their nutrient supply while enriching their husbandry system. Holinger M, Früh B, Stoll P, Kreuzer M, Hillmann E (2018) Livest Sci 218, 50-57. Höne U, Krause E, Bussemas R, Traulsen I, Schrader L (2022) Appl Anim Behav Sci 258, 105821. Høøk Presto M, Algers B, Persson E, Andersson H (2009) Livest Sci 123, 55-62. Klevenhusen F, Hott K, Wiskandt J, Witten S (2026) Livestock Science 305, 105901. Messinger D, Weindl P, Aulrich K, Pleger L, Bellof G (2022) Acta Agric Scand – A Anim Sci 71, 58-73.

Betaine supplementation in adult goats exposed to heat stress conditions

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The MEDGOAT project (PRIMA 2022) addresses the need to adapt goat production systems to global warming in the Mediterranean countries, where animals are usually exposed to heat stress conditions, particularly during the summer season. Therefore, it is imperative to find strategies dedicated to improve the resilience of the animals against heat stress, thus improving health and welfare and also minimizing the loss of animal performance. There is scientific evidence that methionine requirements increase under heat stress conditions. In this sense, betaine, a choline derivative, is important because of its role in the donation of methyl groups to homocysteine to form methionine. Moreover, betaine also demonstrates interesting osmoregulatory properties that might help to mitigate the effects of water restriction. Consequently, our hypothesis was that betaine supplementation helps adult goats to cope with heat stress conditions during the summer season. Therefore, twelve Murciano-Granadina mature goats were divided into two groups of six animals each. The betaine group (BET, n=6) received at 09:30 a.m. every day 8 g of betaine in water solution (20 ml) using a drench gun to ensure a daily intake of approximately 0.2–0.3 g/kg BW, whereas the control group (CTRL, n=6) received daily 20 ml of water. All the animals were kept under pasture conditions (from 10:00 to 17:00, with no water or shadows available), then housed indoors (from 17:00 to 10:00) with free access to fresh water and a diet supplied ad libitum until the next day. The experiment lasted 15 days and animals spent 71% of their daily grazing time (12:00 to 17:00 h) within a Temperature Humidity Index (THI) range of 72 to 78 while exposed to direct solar radiation. Rectal temperature in adult goats increased after the grazing period, exhibiting the BET group a more consistent thermal accumulation, with an increment significantly higher (0.79 °C, p=0.02) than the expected 0.5 °C circadian threshold reported for goats under thermoneutral conditions. In contrast, the increment of rectal temperature in the control group was not significant (0.61 °C; p=0.22) relative to the circadian baseline. No effects on the live weight, biochemical profile, hemogram (including dehydration indicators such as hematocrit or erythrocytes) or ruminal fermentation parameters (including pH and volatile fatty acids profile) were found. Only numerically lower values of beta-hydroxybutyrate in the BET group (P=0.105, not significant) were detected, probably suggesting improved lipid metabolism in the liver. All these results point towards potential osmo-protective effects of betaine, which might have reduced the water evaporation needed to reduce body temperature, thus allowing an adaptative hyperthermia in the BET group. It must be considered, however, that heat stress conditions underwent by these adult goats were moderate/mild, and this would also explain the lack of dehydration observed in both groups. Consequently, further analysis (e.g., expression of genes encoding heat shock proteins) are needed to verify the protective effects of betaine under heat stress conditions.

Effects of live and inactivated yeasts on ruminal pH and redox potential (Eh) in dairy cows under high-carbohydrate challenge

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The objective of this study was to evaluate and compare the effects of live yeast (ADY) and inactivated yeast (IDY) on ruminal physicochemical parameters in dairy cows subject to a high-carbohydrate (HC) challenge. The trial was conducted from September to December 2023 at Schothorst Research Center (The Netherlands) using a 3×3 Latin square design involving 3 dairy cows with cannulated rumen, 3 treatments (Ctrl, ADY, IDY), and 3 consecutive 28-day periods (including 4 phases adaptation, transition, HC challenge and post challenge). The challenge was to raise the diet's starch content from 16.4% to 26.3% of dry matter within 2 days. Ruminal conditions were continuously monitored using measurement system (DASCOR), recording pH and redox potential (Eh) every minute (1 440 datapoints/day). Data of pH and Eh were analyzed using linear mixed models (REML) with treatment and day as fixed factors, cow as a random factor, and hour as a repeated measure. Post hoc comparisons were performed with Tukey's test ($p < 0.05$). Results showed that the increase of rapidly fermentable carbohydrates significantly altered rumen pH and Eh during challenge phase. Yeast supplementations limited the drop in ruminal pH compared with the control group during the last day of challenge phase (Ctrl: 5.86_a; ADY: 5.97_c; IDY: 5.91_b, $p<0.05$). Regarding redox potential, the HC challenge increased Eh by +8.1 mV in control cows during the last day. This result indicating a shift toward a less reductive rumen environment, while IDY mitigated significantly these fluctuations (Ctrl: -292_b mV; ADY: -291_b mV; IDY: -295_a mV, $p<0.05$). In conclusion, inactivated yeasts helped stabilize ruminal environment during a HC challenge. These effects were particularly pronounced in the hours following feeding, suggesting improved resilience of rumen function under high-carbohydrate challenge.

Live yeast supplementation modulates recovery of rumen function and performance following subclinical ruminal acidosis in dairy cows

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Although the impacts of subclinical ruminal acidosis on rumen function and animal performance are well studied, the temporal dynamics of recovery after a challenge and the potential for nutritional strategies to accelerate restoration remain insufficiently characterized. In this study, thirty Holstein lactating dairy cows (≥45 DIM) without any supplement (CTRL) or supplemented with Saccharomyces cerevisiae CNCM I-1077 (LSC) were challenged with a high-starch diet (15.6% CP, 31.0% starch) for 4 weeks. Their recovery trajectories on a conventional diet (15.6% CP, 24.6% starch) were evaluated during the following 4 weeks. Reticulo-rumen parameters and feeding behaviour were continuously monitored using sensors and automated feeding systems, combined with daily milk recordings. A ruminal disorder index (RDi; a validated composite indicator integrating multiple reticulo-rumen pH metrics) was used to reflect ruminal pH disturbances. Recovery dynamics were analysed using mixed-effects models including treatment (CTRL and LSC), days from challenge (linear and quadratic terms), and their interactions. Results showed that RDi, rumination time, and activity exhibited a more dynamic and faster recovery pattern in LSC compared with CTRL cows, characterized by earlier stabilization of ruminal conditions by live yeast. Specifically, LSC cows showed a faster quadratic recovery in RDi following challenge, meaning the reduction rate decreases over time. In contrast, CTRL group followed the opposite pattern with a slower recovery and an increasing reduction rate as days progressed (trt×day: $P<0.05$; trt×day^2: $P<0.05$). Rumination time in LSC cows was associated with a stronger positive day effect combined with more pronounced deceleration ($P<0.05$), whereas CTRL animals recovered more gradually with only mild curvature. Activity displayed a sharper initial decline in LSC cows with stronger negative day effect, followed by a pronounced positive quadratic component ($P<0.05$) in both groups of animals, consistent with the patterns observed for rumination. Although variables related to feeding behaviour and milk composition were impacted during the recovery phase, they did not exhibit biologically strong treatment-specific temporal changes except for total meal duration. Meal duration in CTRL cows showed a steeper initial rise and stronger deceleration and a more negative quadratic term ($P<0.05$), indicating a sharper early increase in CTRL that slowed more quickly compared with the smoother pattern in LSC. Overall, live yeast supplementation significantly modified recovery trajectories of key rumen and behavioural indicators based on reticulo-rumen sensors. These findings highlight the importance of modelling temporal responses when evaluating nutritional strategies and indicate that S. cerevisiae CNCM I-1077 may accelerate functional recovery following subclinical ruminal acidosis.

Synergistic Co-fermentation of Palm Kernel Cake Enhances Nutrient Digestibility and Modulates Rumen Microbiota in Beef Cattle

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This study posits that co-fermenting palm kernel cake (PKC) may enhance its nutritional value, and that the partial substitution of conventional feed with this fermented product could improve nutrient digestion in beef cattle by positively influencing the rumen microbiota. To test this, PKC was subjected to a synergistic co-fermentation process employing Aspergillus niger and mannanase. Thirty beef cattle were randomly assigned to two groups (n=15): a control group (CON) receiving a standard basal diet, and a treatment group (FP) where fermented PKC (FPKC) partially replaced soybean meal and corn. Throughout a 90-day feeding trial, body weight, rumen fluid, and fecal samples were collected on days 30, 60, and 90 to evaluate growth performance, rumen fermentation parameters, and nutrient digestibility. Furthermore, rumen fluid samples collected on day 90 underwent 16S rRNA gene sequencing to analyze the microbial community structure. The results indicated that the synergistic co-fermentation involving fungi and enzymes effectively enhanced the nutritional profile of PKC, indicated by a significant reduction in mannan and acid detergent fiber (ADF) contents, coupled with a notable increase in crude protein (CP) content ($P < 0.05$). In a feeding trial, no significant differences in growth performance were observed between the FP and CON groups ($P > 0.05$). However, after 90 days, the FP group showed significantly better digestibility of dry matter (DM), CP, and neutral detergent fiber (NDF) compared to the CON group ($P < 0.05$). Rumen fermentation analysis revealed that the FP group had lower levels of butyrate and NH_3-N compared to the CON group ($P < 0.05$). Microbial 16S rRNA sequencing revealed a significant alteration in the rumen microbiota composition. The FP group exhibited a higher relative abundance of Bacteroidetes, while the CON group was predominantly composed of Firmicutes. The Rikenellaceae RC9 gut group, a genus renowned for its ability to degrade complex structural carbohydrates and enhance fiber fermentation efficiency, emerged as a key marker for the FP group. In contrast, the CON group was characterized by a predominance of Ruminococcus, Christensenellaceae R-7 group, and Butyrivibrio. Collectively, these findings demonstrate that the synergistic co-fermentation process efficiently degrades complex antinutritional carbohydrates such as mannan while simultaneously enhancing the protein content of PKC. Administering FPKC to beef cattle enhanced nutrient digestibility and positively altered the rumen microbiome to support fiber-degrading bacteria. This highlights the potential of fermented palm kernel cake as an effective feed ingredient for improving rumen health and nutrient use in beef cattle.

Exploratory investigation of maternal care and early postnatal calf behavior related to milk intake in a dam-bound rearing system in the first 24 hours after birth

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Maternal behavior, including intensive licking and encouraging the calf to suckle immediately after birth, might be essential for activating the newborn's physiological processes and aiding thermoregulation by reducing potential evaporative heat loss. Although suckling at the udder within the first 24 hours is a critical component of neonatal development in cow-bound rearing systems, this behavior and influencing factors remains insufficiently described in detail. The aim of this exploratory study was to quantify key maternal and calf behavioral patterns occurring after birth, with a particular focus on behaviors associated with early colostrum intake. In addition, the study aimed to evaluate how maternal behavior, cow- and calf-related factors, and environmental conditions influenced the calf's colostrum intake during the first 24 hours of life. The study was conducted at the Veitshof research farm of the Technical University of Munich using data from cows (n = 13) and their calves during the first 24 hours after birth. Cow-related data included parity, gestation length, calving duration, and udder morphology, the latter assessed according to breed value estimation scores for Brown Swiss cows. In addition, maternal behavior was video recorded and evaluated, including the duration and intensity of licking as well as the location of licking events on the calf's body, which was divided into five anatomical regions. Calf-related measurements comprised body weight, body temperature at birth and after 24 hours, and a detailed description of behavioral aspects such as activity, inactivity, udder-searching duration (reflected the time from birth until the calf successfully located the udder), drinking duration (colostrum intake time), and drinking frequency. Furthermore, climate conditions were recorded, and the temperature-humidity index was calculated. Statistical analyses included descriptive statistics and Spearman rank correlations. The results showed that cows licked the calves on 122 ± 11 (mean ± SE) events with a total duration of 122.28 ± 9.24 minutes in the first 24 hours after birth. Ten out of 13 calves suckled colostrum from the udder within the first 6 hours postpartum, with an average drinking duration of 6.43 ± 1.87 minutes in this period. Three calves required human assistance (teat finding) to obtain colostrum from the udder. Over the first 24 hours, calves spent on average 30.36 ± 7.32 minutes searching for the udder and 40.12 ± 4.98 minutes drinking. The mean number of drinking events during the first 24 hours was 19 ± 3. Correlation analysis indicated that calves spending more time searching for the udder were generally more active ($r = 0.52$, $p \leq 0.05$) and experienced a higher number of maternal licking events ($r = 0.36$, $p \leq 0.05$). Udder height over ground showed a weaker association with udder-search duration ($r = 0.24$, $p \leq 0.05$). Total drinking duration was positively linked to calf activity ($r = 0.37$, $p \leq 0.05$), while higher parity of the dam ($r = –0.29$, $p \leq 0.05$) and longer teats ($r = –0.23$, $p \leq 0.05$) were associated with reduced drinking duration. Overall, the findings highlight the importance of integrated management strategies such as continuous observation to support successful colostrum intake in cow–bound rearing systems.

The effect of 3-sialyllactose in combination with Bacteroides thetaiotaomicron isolated from Ningxiang pigs on the intestinal development of piglets

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Intestinal development is a core process in the rapid growth of piglets, and optimizing gut microbiota homeostasis during the early-life window is critical for improving growth performance. Milk oligosaccharides are key bioactive components in maternal milk, playing important roles in promoting probiotic colonization, maintaining microbial balance, and regulating host immunity. Our previous results revealed significant differences in milk oligosaccharide profiles and gut microbiota structures between Chinese indigenous Ningxiang (NX) and Large White sows. A high SL content in NX colostrum was significantly positively correlated with the abundance of Bacteroides thetaiotaomicron (BT) in piglets. The BT2 strain isolated from Ningxiang pigs efficiently metabolized sialyllactose (SL), particularly 3-SL, through GH enzymes, producing sialic acid and SCFAs. Therefore, this study further explored the effects of this key bacterial strains utilizing specific oligosaccharides (3-SL) on piglets intestinal development and function. Six litters of cross-bred (Duroc × Landrace× Yorkshire) piglets with similar genetics and parity were selected. For each litter, three healthy newborn piglets with similar body weight were orally administered saline, 3-SL, or 3-SL+BT2 from day 1 until weaning at day 21, followed by sampling on day 24. The results showed that, compared to the control group: (1) The 3-SL and 3-SL+BT2 groups had significantly reduced diarrhea rates ($P < 0.05$). (2) Villus height (VH) and villus height to crypt depth ratio (V/C) in the small intestine were significantly increased, while crypt depth (CD) in the small intestine was significantly decreased, and CD in the colon was significantly increased in both 3-SL and 3-SL+BT2 groups ($P < 0.05$). (3) Serum D-lactic acid (D-LA) levels were significantly reduced in the 3-SL and 3-SL+BT2 groups ($P < 0.05$). Intestinal development- and barrier-related gene and protein expression in the ileum and colon were significantly up-regulated, and the levels of sialic acid, acetate, propionate, and total short-chain fatty acids (SCFAs) in the colonic contents were significantly elevated ($P < 0.05$). (4) In the 3-SL+BT2 group, butyrate and valerate levels in the colonic contents were significantly increased compared to the control group($P < 0.05$). Compared to the 3-SL group: (1) The diarrhea rate was even lower in the 3-SL+BT2 group. (2) Crypt depth in the duodenum was significantly lower, V/C was significantly higher, and the number of AB-PAS-positive cells was significantly increased with more intense staining in the 3-SL+BT2 group ($P < 0.05$). (3) The contents of sIgA were significantly increased, and the expression of gut development and barrier-related genes or proteins such as β-catenin, TGF-β, IGF1, Lgr5, and ZO-1 was significantly up-regulated ($P < 0.05$). The results indicate that the combination of 3-SL and BT2 effectively promotes intestinal development and barrier function in piglets, thereby improving gut health. These findings provide a theoretical basis for the development and application of synbiotics and offer a reference for nutritional interventions aimed at improving piglet intestinal health.

Investigating the optimal histidine to lysine ratio in piglets: impact on performance, blood parameters and carnosine content in the skeletal muscle

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Histidine (His) is an essential amino acid in pigs; therefore, an inadequate supply of His may impair growth performance. However, research on His requirement of pigs is scarce. In addition to being involved in protein synthesis, His has broad functions and is incorporated into specific body proteins, which may should be considered when estimating its requirement. Histidine is an important component of haemoglobin (Hb), and His deficiency may influence Hb synthesis. Furthermore, His can be metabolized together with ß-alanine to form the dipeptide carnosine, which is an important antioxidant. The aim of this study was to determine the optimal standardized ileal digestible (SID) His-to-Lys ratio in nursery piglets for growth, carnosine concentration in muscle, and selected blood parameters. 192 piglets (Topigs TN70; starting weight: 7.2 kg) were placed in 8 pens/treatment (4 pigs/pen; 50/50 castrated/female piglets). A diet based on cereals and soybean meal was formulated to be deficient in His. Supplementary L-His was used to achieve six graded levels of SID His-to-Lys ratios (0.20, 0.24, 0.27, 0.31, 0.35 and 0.38, respectively). Body weight (BW) and feed intake (FI) were measured at start and end of trial. Feed conversion ratio (FCR) was calculated. At the end of trial (day 41), 72 animals were euthanized and sampled to measure carnosine in the M. longissimus dorsi. Additionally, blood plasma was collected from 72 animals for the analysis of Urea-nitrogen (PUN) and the small blood count parameters (haematocrit, haemoglobin, leucocytes and thrombocytes, respectively). A one-way ANOVA with the fixed effects of treatment and sex was applied to the data. Starting BW was used as a covariate. Means were compared using Tukey test ($P<0.05$). A quadratic regression model was applied to determine SID His-to-Lys ratio needed to achieve maximum performance to be considered the recommended His-to-Lys ratio. The recommended SID His-to-Lys ratios (0.33, 0.34 and 0.34) were estimated for BW, FI and FCR. Haematocrit, Hb and PUN showed a quadratic response with a recommended SID His-to-Lys ratio of 0.32, 0.33 and 0.33, respectively. With a dietary SID His-to-Lys ratio of 0.20 and 0.24, the measured Hb values (9.8 g/dL and 10.1 g/dL, respectively) were below the laboratory reference range (10.8 g/dL to 14.8 g/dL). The lowest carnosine content (70.0 mg/100g of meat) was observed at SID His to Lys ratio of 0.20 and increased linearly to a maximum of 383.4 mg/100 g of meat at SID His to Lys of 0.38. The SID His-to-Lys ratio of 0.34 could be suggested for a maximum performance in piglets, with 0.34 being also recommended by NRC. The muscular carnosine concentration increased linearly with increasing dietary His without reaching a plateau. Animals fed with the two lowest SID His-to-Lys ratios (0.20 and 0.24) had the lowest Hb levels, which may indicate anaemia in the animals. Although it is well described that piglets may develop anaemia with a reduced Hb value due to iron deficiency, the finding that a dietary His deficiency can also lead to anaemia is new and requires further research.

Effects of dietary carob pulp as a source of non-starch polysaccharides on carcass traits and fatty acid composition in intensively reared Iberian crossbred pigs

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A total of 60 Duroc x Iberian pigs (109±2.1 kg of body-weight, BW; 8.5 months of age), half surgically castrated males (SCM) and half immunocastrated females (ICF), were selected from six split-sex large pens (2 m2/pig). The aim of the study was to evaluate the impact of dietary carob pulp (CPULP) inclusion (0, 10 and 20%), as a source of non-starch polysaccharides (NSP), during the last 50 days of the finishing phase on growth, carcass quality and fat composition. Each pen received ad libitum one of the three experimental diets differing in CPULP level. The three diets were formulated to provide similar nutrients; 2,500 kcal net energy/kg, 110 g crude protein/kg and 5.2 g total Lysine/kg. However, increasing CPULP inclusion raised NSP content (211, 228 and 271 g/kg of feed, respectively). This was compensated with adding lard, thereby increasing per kg of feed the supply of saturated fatty acids (16, 21 and 26 g SFA/kg) and monounsaturated fatty acids (17, 23 and 30 g MUFA/kg) with minimal changes in polyunsaturated fatty acids (15, 15 and 16 g PUFA/kg). The BW was recorded the first day of the trial and the day before the slaughter to calculate average daily gain (ADG). Pigs were slaughtered in a commercial abattoir (30 km away) with a 20-h lairage period. Carcass weight (including head) was registered to calculate killing-out proportion. The weight of trimmed hams and shoulders was recorded 24 h post-mortem, and yields were expressed as a proportion of carcass weight. The loin pH (pH45) was measured 45 min post-mortem (last thoracic rib) and the subcutaneous lumbar fat thickness was also measured. Finally, fatty acid composition of subcutaneous fat was analyzed. The data were statistically analyzed with standard using least square means models with diet, sex and their interaction as fixed effects. Dietary CPULP inclusion did not affect (P>0.10), final BW (152.2, 151.7 and 152.4±2.8 kg), ADG (799, 801 and 760±26.6 g/day), carcass weight (120.9, 121.6, 122.3±2.3 kg), or killing-out proportion (79.5, 80.1 and 80.2±0.44%). However, pigs fed 0% and 10% CPULP showed thinner fat thickness than those fed 20% CPULP (5.3, 5.4 vs. 6.0±0.17 cm, P<0.01). The CPULP inclusion did not affect (P>0.10) ham yield (10.4, 10.3 and 10.2±0.23%) or shoulder yield (6.6, 6.3 and 6.6±0.14%). However, CPULP decreased pH45 (6.56 vs. 6.38 and 6.44±0.038, P<0.05). The contents in SFA (38.7, 37.8 and 38.1±0.31%) and MUFA (52.8, 53.0 and 52.8±0.30%) of fat did not differ among diets (P>0.10) but, unexpectedly, PUFA content increased with CPULP inclusion (8.4 vs. 9.2 and 9.1±0.12%, P<0.001). Sex did not affect ADG (765 vs. 808±21.8 g/day, for SCF and ICF; P>0.10). However, SCM were lighter at slaughter than ICF (147.6 vs. 156.6±2.3 kg; P<0.01), and it was also detected in carcass weight (117.6vs. 125.5±1.9 kg, P<0.01). However, killing-out proportion did not differ between sexes (79.7 vs. 80.1±0.36%, P>0.10) nor fat thickness (5.6 vs. 5.6±0.14 cm for SCM and ICF, P>0.10). Although sex did not influence ham yield (10.1 vs. 10.5±0.20%, in SCM and ICF, P>0.10), shoulder yield was greater in SCM than in ICF (6.7 vs. 6.3±0.12%, P<0.05). Loin pH45 was similar between sexes (6.44 and 6.48±0.031, in SCM and ICF, P>0.10) and fatty acid composition was likewise unaffected by sex. In conclusion, dietary inclusion of 20% CPULP increased carcass fatness and PUFA content without impairing growth rate in intensively Iberian crossbred pigs, regardless of sex. A moderate inclusion level of 10% CPULP increased PUFA content but had minimal effects on carcass fatness.

Integrating growth performance and tissue functional transcriptomics to elucidate the effects of a xylanase and β-glucanase combination in post-weaning pigs

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The aim of the study was to investigate the impact of xylanase and β-glucanase (XB) dietary supplementation on piglet performance and tissue specific functional transcriptomic responses when fed diets differing in NSP content. Our hypothesis was that supplementation with XB to diets fed during the first two weeks post-weaning improves piglet performance and metabolic efficiency, and attenuates the physiological challenge caused by high dietary NSP content. Eighty entire male weaned pigs across five batches ((LR × LW) × Duroc; 8.6 ± 0.42 kg BW; 28 days of age) were assigned to four dietary treatments in a 2 × 2 factorial arrangement with two NSP level (LNSP vs. HNSP) and with or without XB supplementation as main factors. Diets were formulated to be isoenergetic and balanced for digestible amino acids. The LNSP diet was a corn -soya based diet, whereas the HNSP diet included barley, wheat, wheat bran, and rapeseed meal (analyzed total NSP: 7.8 vs. 12.0%, respectively). Piglets were fed diets in mash form the first 14 days post-weaning. Body weight, feed intake, and feed conversion ratio (FCR) were individually recorded. On day 14 all pigs were euthanized and stomach, ileum, and liver tissues were collected for RNA extraction. The mRNA was sequenced (Illumina, 150 PE, poly-A enriched, 20M reads per sample) and gene abundance calculated using Kallisto. Differential gene expression (DGE) was tested among treatments (Deseq2), followed by a topology-based Quantitative Pathway Activation method (QPA, Biofractal, Portugal) using an adjP<0.05 threshold. High NSP increased FCR (+13%; $P < 0.05$). In contrast, XB supplementation increased average daily feed intake (+14%; $P < 0.05$) and average daily gain (+29%; $P < 0.01$) and tended to reduce FCR (-9%; $P < 0.1$), independent of NSP level ($P > 0.1$). Transcriptomic analysis revealed that NSP level was the primary driver of hepatic DGE changes, whereas XB supplementation on HNSP diets predominantly affected gastric and ileum responses. Pathway level analysis showed that increasing NSP induced adaptive responses in the stomach and ileum, characterized by more active tissue remodeling, barrier function, and immune sensing, and promoted coordinated nutrient metabolism activation in the liver. These responses indicate a developmental stimulus that increased physiological demand and a mechanistic link to the increased FCR observed in pigs fed HNSP diets without XB. Conversely, XB supplementation attenuated NSP-induced adaptive response in the stomach and ileum under HNSP conditions, reducing immune recognition and innate activation while preserving tissue integrity, and markedly inhibited hepatic cellular stress and inflammatory responses. Under LNSP conditions, XB promoted a metabolically efficient phenotype across all three tissues, activating digestive, absorptive, and antioxidant pathways in the ileum, supporting hepatic and gastric mucosal development, without inducing compensatory stress responses. Notably, BPIFA1 expression was downregulated in pigs fed LNSP diets supplemented with XB (Padj < 0.01), consistent with reduced epithelial stress and microbial signaling at the intestinal mucosa. These findings demonstrate that while dietary NSP primarily elicits compensatory physiological responses that may limit efficiency, XB supplementation improves feed intake and performance by promoting more efficient tissue specific functional states across both low and high NSP diets.

Effects of dietary γ-aminobutyric acid supplementation on growth performance and novel object test responses in nursery piglets

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Weaning is a critical developmental stage in pigs, and implementing effective nutritional strategies during the nursery period can enhance growth performance in the subsequent fattening phase. Recent studies have explored the use of dietary supplementation γ-aminobutyric acid (GABA), an inhibitory neurotransmitter in the central nervous system, which may influence the GABAergic system and the gut–brain axis, reducing the adverse effects of stress and improving gastrointestinal function. This study aimed to investigate the effects of GABA supplementation on performance and behavioral responses to fear in weaned piglets, as assessed through a novel object test. A total of 128 piglets (Duroc x Landrace) at 28 d-old were allocated into 16 pens (8 pigs/pen) at the UAB experimental farm. At arrival, pens were assigned to a control diet (CTR; 6.9 ± 0.96 kg) or supplemented with 100 mg GABA /kg of feed (GABA; 6.9 ± 0.75 kg), balanced by weight between treatments. At days 21, 32 and 35 a back test was conducted to identify piglets' coping style as high resistance (HR) or low resistance (LR). Body weight (BW), average daily gain (ADG) and average daily feed intake (ADFI) per pen was measured weekly. At 47 d-old, a total of 20 piglets (10 HR and 10 LR), balanced across treatments, were individually subjected to a novel object test (NOT) in their familiar pen, including 1 minute of habituation followed by 3 min with the exposition to the novel object (NO). Latency to touch the NO, as well as the duration and frequency of contact with the NO, were measured. GABA-supplemented piglets tended to exhibit higher ADFI compared to CTR piglets during the first (136 vs. 113 g/day; P = 0.069) and third weeks (447 vs. 417 g/day; P = 0.076) post-weaning. ADG was higher in GABA group than CTR during the first week post-weaning (72 vs. 32 g/day; P = 0.041), with a tendency to a higher ADG in the second week (176 vs. 127 g/day; P = 0.079), and from 35 d-old onward, GABA-supplemented piglets had higher BW compared with CTR (Δ = 600g; P < 0.05). Regarding the NOT, no differences were observed in the interaction between treatment and coping style, and no difference were observed in the habituation phase. GABA-supplemented piglets touched the novel object more quickly (23.6 vs. 140.4 s; P < 0.001), more frequently (9.9 vs. 5.8 n; P = 0.007), and for longer durations (31.7 vs. 13.5 s; P = 0.025) than the CTR group. HR-piglets showed a higher time touching the NO (32.2 vs. 12.9 s; P = 0.022) than the LR-piglets. The potential of GABA to reduce stress and enhance intestinal health could be observed in piglets, as it may have enabled them to better cope with weaning stress and, consequently, prevent the marked reduction in feed intake and performance observed in CTR pigs. Moreover, the stress- and anxiety-reducing potential of GABA is reflected more clearly in the results of the NOT, were pigs reacted with less fear to the NOT as reflected by their faster contact to the NO and the longer time spent interacting with it. However, the treatment did not appear to alter the inherent responses associated with each pig's coping style. In conclusion, GABA supplementation enhanced performance during the early post-weaning period and reduced fear responses in the NOT, without altering inherent coping styles, suggesting improved emotional state and welfare in nursery pigs.

Magnesium-protected zinc as an alternative to zinc oxide on growth of weaned piglets

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Zinc is an essential trace element in piglet nutrition, playing a key role in growth, immune function, gut and overall health. However, the amount of zinc naturally present in standard feed is insufficient to meet the animals' requirements, making supplementation critical, particularly during the post-weaning period when piglets are most vulnerable. Conventional zinc sources, such as zinc oxide (ZnO), are widely used but can have limited bioavailability, which may reduce their effectiveness in supporting optimal growth and health. This limitation has driven the design of innovative zinc sources, including magnesium-protected zinc. By enhancing zinc bioavailability and absorption thanks to a patented magnesian protection, this novel source helps ensure that piglets receive the levels needed to support their development, ultimately leading to better zootechnical performance and supported gut health. A 42 day study was carried out to evaluate the growth performance of post-weaning piglets in a controlled feeding trial. Twenty-four castrated male piglets, weaned at 28 days of age (initial body weight 4.7 ± 0.45 kg), were randomly allocated to two experimental treatments, with four pens of three animals per treatment. The trial consisted of a 14-day starter phase followed by a 28-day grower phase. Two diets were formulated to provide a total zinc level of 150 mg/kg feed, differing only in the zinc source: conventional 125 ppm zinc from zinc oxide (CON) or 125 ppm zinc from magnesium-protected zinc (CAPMAG® Zn; CAP). Diets were based on wheat, barley, and soybean meal and were distributed as crumble during the starter phase and as pellets during the grower phase, with ad libitum access. Body weight, average daily weight gain (ADG), average daily feed intake (ADFI), and feed conversion ratio (FCR) were recorded to evaluate growth performance. Data were analyzed using a linear model (R software) using weaning weight as a co-variable and with the pen as the unit. This analyze was followed by Tukey test. During the starter phase, ADG for the CAP group was 144 g/day compared with 125 g/day for CON (+15.3%; $P>0.05$). ADFI was also higher but non-significant in CAP (226 vs. 179 g/day; +26.1%; $P>0.05$), leading to a non-significant increase in FCR (1.651 vs. 1.441; $P>0.05$). In the grower phase (D14–42), ADG was significantly improved by +24.3% for CAP compared to CON (389 vs. 313 g/day; $P<0.05$). Feed intake tended to be higher (645 vs. 559 g/day; +15.3%; $P>0.05$) and FCR showed numerical reduction for CAP versus CON (1.658 vs. 1.568; $P>0.05$). Over the entire post-weaning period, CAP piglets showed a significant higher ADG by +22.8% compared to CON (307 vs. 250 g/day; $P<0.05$) and an improved final body weight by +2.34 kg (17.62 vs. 15.27 kg; +15.4%; $P>0.05$). ADFI was slightly increased (505 vs. 433 g/day; +16.8%; $P>0.05$), and overall FCR improved numerically (1.645 vs. 1.727; −4.8%; $P>0.05$). These results demonstrate that magnesium-protected zinc enhances growth performance and feed efficiency under challenging post-weaning conditions, even with few replicates and animals per pen. The most pronounced effects occurred during the grower phase and over the whole post-weaning period, highlighting the improved bioavailability of this novel form of zinc. Further studies are needed to clarify its mode of action. In conclusion, supplementing post-weaning piglets with magnesium-protected zinc improves growth compared with conventional ZnO at the same inclusion level, offering a practical approach to optimize zinc nutrition during this critical period.

Microalgal Chlorella sorokiniana as a functional feed ingredient improves post-weaning piglet performance

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The post-weaning phase in swine production is marked by pronounced metabolic and immunological challenges that can impair growth and increase vulnerability to enteric disturbances. This study combined cellular bioactivity screening with controlled in vivo trials to assess the potential of Chlorella microalgae as a functional dietary component to support piglet performance during this critical period. In vitro characterization was performed using receptor-activity profiling in HEK293 cells and transcriptomic analysis in IPEC-J2 porcine intestinal epithelial cells to compare Chlorella sorokiniana and Chlorella vulgaris. Across both platforms, C. sorokiniana exhibited a stronger and broader bioactive profile than C. vulgaris. The carbohydrate-enriched "rest fraction" of C. sorokiniana particularly modulated pathways associated with innate immunity, antiviral defense, and metabolic regulation, including mTOR-related signaling. Based on these results, two in vivo trials were conducted to determine the optimal inclusion level and processing method for C. sorokiniana in weaned piglets. In the first phase (day 1–8 post-weaning), dietary inclusion of 0.8% drum-dried C. sorokiniana significantly improved growth performance compared to the control group. Specifically, piglets receiving 0.8% microalgae achieved a higher body weight at day 8 ($P < 0.05$) and an increased average daily gain (ADG) during the first week ($P < 0.05$). Feed efficiency was also significantly enhanced ($P < 0.05$) in the 0.8% inclusion group. Further investigations into processing methods showed that while mechanical cell wall rupture (bead-milling) resulted in a numerical improvement in feed conversion ratio (FCR) compared to intact cells, the difference did not reach statistical significance ($P > 0.05$). Increasing the inclusion level from 0.8% to 1.6% did not yield further significant improvements in ADG or FCR ($P > 0.05$), indicating a plateau in biological response at the 0.8% level. Regarding intestinal health, no significant differences were observed in faecal consistency scores between the control and algae-supplemented groups throughout the study ($P > 0.05$). Additionally, the performance benefits observed in the first week did not persist into the late rearing phase (day 14–35), where growth parameters were statistically similar across all treatments ($P > 0.05$). In conclusion, C. sorokiniana is a bioactive functional ingredient that effectively mitigates the immediate post-weaning performance dip. The study demonstrates that a dietary inclusion of 0.8% drum-dried C. sorokiniana significantly boosts growth and feed efficiency during the first week post-weaning, supported by strong in vitro evidence of immune-metabolic pathway activation. These findings suggest that microalgae supplementation is most effective when targeted at the critical transition window immediately following weaning.

Tiny but mighty: small dose of standardized dry grape extract potentiates antioxidant defenses in broiler chickens

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Antioxidant defenses are crucial for livestock resilience against many stressors. Over the last few decades, a strong emphasis has been put on potential complementary antioxidant from natural sources. They are however often studied in vitro, or at high concentrations. Thus, the aim of the present study was to evaluate the effect of a dietary supplementation of broiler chickens with a commercial standardized dry grape extract (NG) on zootechnical performances and physiological antioxidant parameters. 756 day-old all-male Ross 308 broiler chickens were randomly divided into two groups and raised for 35 days. Control birds (CTL, n=392) received a classic feeding program. The rest of the chickens (NG, n=364) were fed the same dietary program with the addition of 30ppm of NG (Nor-Grape®, Nor-Feed, France) from D1 to D35. Body weight, feed intake and FCR were measured for each replicate per period. Blood samples were collected on 30 chickens per group on D11 and D35 to measure global antioxidant protection (TEAC) and oxidative damages (MDA). Endogenous antioxidant levels were also monitored to assess the potential modulatory effect of the supplementation on CAT, SOD and GSH-Px. On D35, mean liveweight of NG chickens was greater than the CON (+3.1%, $p<0.001$). No differences ($p>0.05$) were found in terms of feed intake or mortality between groups, apart from a lower FCR in the finisher phase for NG broilers ($p<0.05$). On D11, antioxidant defenses were positively modulated by the supplementation as evidenced by a higher TEAC (+28.4%, $p<0.001$), CAT and SOD activities (+27.9% and +8.3% respectively $p<0.05$) compared to CTL birds. On D35, NG birds showed global and significant increase in all antioxidant defenses monitored (+3.3% TEAC, +15.9% CAT, +16.7% GSH-Px and +9.0% SOD, $p<0.01$) parallel to a significant reduction in oxidative damages (-14.9%, $p<0.05$). Overall, these results confirm previous findings showing that a dietary addition of 30ppm of NG potentiates the antioxidant defense system, improving the chickens' resilience. This translates into improved performances as illustrated in this work.

Protective antioxidant effects of a dietary polyphenol combination in broilers under heat stress

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In livestock, heat stress impairs nutrient digestibility, growth, and muscle development, increasing maintenance energy requirements and reducing feed efficiency and performance while increasing meat quality defects. Citrus flavonoids and curcuminoids mitigate cellular oxidative stress mainly through the upregulation of endogenous antioxidant enzymes via Nrf2–Keap1, inhibition of ROS-producing enzymes, and direct radical scavenging. This study aimed to assess the antioxidant protective effects of a dietary combination of citrus flavonoids and curcuminoids in broiler chickens under heat stress, focusing on performance, oxidative status, carcass traits, and meat oxidative quality. A 42-day trial was conducted with 600 male Ross 308 AP95 broilers assigned to five dietary treatments (10 pens/treatment; 12 birds/pen): negative control (NC), positive control (PC; +100 IU/kg vitamin E), and the NC diet supplemented with a combination of citrus flavonoids and curcuminoids (TNP; HEBOX®) at 300, 600, or 900 mg/kg. Birds were reared in floor pens and exposed to heat stress from days 28 to 42 (mean temperature–humidity index >29). At day 42, growth performance, carcass traits, and oxidative status were evaluated by measuring malondialdehyde (MDA), paraoxonase-1 (PON1), and total antioxidant capacity (TAOC) in plasma, and malondialdehyde (MDA) in thigh meat, using 4 birds/pen (n=40). Results are expressed as mean ± SEM and analysed by one-way ANOVA by SAS. TNP supplementation tended to increase body weight ($P<0.05$ ANOVA trend test), by 3% at 900 mg/kg (2811±38 g) vs. NC (2728±55 g). Mortality, average daily feed intake and feed conversion ratio numerically improved with increasing TNP inclusion. TNP tended to increase yield (761.7±1.9 g/kg TNP900 vs. 755.5±2.7 g/kg NC) and breast relative weight (272.0±2.0 g/kg TNP900 vs. 269.1±2.5 g/kg NC) in a concentration-dependent manner ($P<0.05$ ANOVA trend test), and the relative weight of drumsticks were significantly increased in TNP600 ($P<0.05$; 90.0±0.6 g/kg) vs. NC (87.5±0.6 g/kg) and PC (87.4±0.6 g/kg). At 600 and 900 mg/kg, TNP decreased MDA in plasma by 14% ($P<0.05$; 10.20±0.34 nmol/mL and 10.31±0.41 nmol/mL, respectively) vs. NC (12.05±0.22 nmol/mL), with no effect of PC. TAOC of plasma increased by 8.7% ($P<0.05$) in TNP600 (0.841±0.023 mM) vs. NC (0.774±0.009 mM), and tended to be higher in TNP900, vs. NC and PC. PON1 showed the lowest level in NC (16.20±0.41 ng/mL) and raised significantly in PC (20.62±0.39 ng/mL) and with TNP, with the highest value at 300 mg/kg (24.63±0.55 ng/mL). PC did not reduce meat MDA (9.22±0.26 µg/g) vs. NC, whereas TNP at 600 mg/kg reduced MDA by a 10.5% and 15.7% vs. NC (9.59±0.39 µg/g) and TNP300 (10.18±0.41 µg/g), respectively ($P<0.05$). This study indicates that dietary supplementation with TNP enhances the capacity of broilers to cope with heat challenge by improving oxidative balance and associated metabolic responses. Improvements in plasma MDA, PON1, TAOC demonstrate the in vivo antioxidant effects of TNP under heat stress and its support of endogenous antioxidant defenses. This antioxidant protection improved meat oxidative stability, as evidenced by reduced MDA levels in thigh meat, performance, mortality, and carcass traits, suggesting a more efficient metabolic adaptation to thermal challenge. In conclusion, dietary combination of citrus flavonoids and curcuminoids represents an effective nutritional strategy to mitigate oxidative imbalance and support performance, carcass quality, and meat oxidative stability in broiler production systems exposed to heat stress.

From agricultural residue to high-value prebiotics: Upcycling sorghum lignocellulosic biomass into cello-oligosaccharides

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The development of functional feed ingredients that selectively modulate gut microbiota is central to next-generation animal nutrition strategies aimed at improving animal health and productivity. Cello-oligosaccharides (COS) are emerging prebiotics; however, sustainable production of structurally and functionally defined COS remains limited. This study developed structurally defined COS from sorghum crop residue and evaluated their prebiotic functionality. Sorghum crop residue was subjected to alkaline fractionation using sodium hydroxide (2.0–3.5 M) to obtain a cellulose-enriched substrate for enzymatic bioconversion in COS. Pretreatment with 2.0 M sodium hydroxide achieved the highest cellulose recovery (35.6% yield; 94.5% recovery). FTIR confirmed effective delignification and hemicellulose removal. Scanning electron microscopy (SEM) revealed substantial disruption of the compact lignocellulosic matrix and increased surface porosity following alkali treatment, facilitating enzyme accessibility. X-ray diffraction (XRD) analysis indicated enhanced cellulose crystallinity, with the crystallinity index increasing from 73.58% in untreated residue to 81.38% following 2.0 M NaOH treatment. Based on recovery efficiency, structural integrity and reduced chemical severity, 2.0 M NaOH was selected as the optimal pretreatment condition. The recovered cellulose was subsequently subjected to controlled enzymatic depolymerisation using Aspergillus niger cellulase to selectively produce COS. A central composite design was employed to assess the effects of pH (4.5–5.5), temperature (30–50 °C), enzyme loading (20–30 IU g^{-1}) and incubation time (8–24 h). Optimised reaction conditions (pH 4.5, 30 °C, 30 IU g^{-1} enzyme loading and 8 h incubation) resulted in the production of COS. HPLC analysis revealed 40.33% COS (DP2–DP5) and 29.28% glucose from 2% (w/v) cellulose. Notably, cellohexaose (DP6) was additionally detected, increasing the total COS fraction to 58.41 % with 30.76 % glucose. COS production followed a quadratic model, while glucose formation was primarily influenced by enzyme loading and incubation time. Prebiotic functionality was assessed based on selective microbial growth, using Ligilactobacillus salivarius RBL22 and Escherichia coli ATCC 25922. COS exhibited a higher prebiotic activity score (1.25) and prebiotic index (1.80) compared with standard inulin (PAS-1.13; PI 1.59) and native cellulose (PAS 0.52; PI 0.31), indicating superior selectivity towards beneficial bacteria. Overall, this study demonstrates that alkali-assisted structural modification of sorghum lignocellulose, validated through FTIR, XRD and SEM analyses, enables the production of functionally superior COS as a sustainable prebiotic in animal feed application.

EntoBoost: a multi-model screening platform to identify and study insect fractions with health-promoting effects in animal feeds

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Inclusion of insect-derived ingredients in livestock diets has been associated with health benefits across diverse production systems. However, as with many other promising functional feed ingredients, these effects have proven difficult to reliably reproduce and the underlying mechanisms remain poorly understood. Screens for bioactivity in natural products typically measure antimicrobial and/or antioxidant activity but moving directly from these readouts to in vivo trials with production animals is risky and the success rate is low. To streamline the development of functional feed ingredients, there is a need for intermediate screening platforms that model additional biological complexity while remaining simple enough to evaluate multiple candidates. As part of the EntoBoost project, we aim to address this knowledge and methodology gap using complementary fractionation protocols and bioactivity assays designed to disentangle the effects of complex insect-derived feed ingredients through systematic evaluation of black soldier fly (BSF; Hermetia illucens) larval fractions. BSF larvae are processed via a combination of wet mode fractionation (press cake, pellet, aqueous fraction, cream, and lipid) and chemical purification (proteins, lipids, and chitin) to produce a library of potentially bioactive insect fractions. These fractions are then screened using complementary infection models: (1) for broilers: an in vitro ileal fermentation model challenged with NetB-producing Clostridium perfringens; (2) for pigs: an analogous model using enterotoxigenic Escherichia coli (ETEC); and (3) for shrimp: an in vivo Artemia franciscana model challenged with Vibrio spp. to assess pathogen inhibition and survival. The broiler model has been established and validated. Grower feed was subjected to in vitro digestion using a broiler-adapted INFOGEST protocol with gastric and intestinal phases at 41 °C. The resulting digest served as substrate for ileal fermentation, inoculated with contents from two-week-old broilers and spiked with C. perfringens strain CP56 at approximately 3 log CFU/mL. A qPCR assay targeting the netB toxin gene confirmed stable colonization throughout 24-hour fermentation. Infected fermentations exhibited elevated gas production and reduced acetate levels relative to non-infected controls, indicating measurable shifts in microbial metabolic activity under pathogen challenge. Acetate was the sole detectable short-chain fatty acid, consistent with the ileal origin of the inoculum. This validated platform now serves as the basis for systematic screening of BSF fractions for anti-infective activity. Screening of chitin-derived fractions is currently underway across all three platforms, and initial results from the broiler, pig, and Artemia models will be presented. The combination of systematic fractionation with species-specific infection models creates a pipeline to link specific bioactive components to measurable anti-infective outcomes. While developed here for insect-derived fractions, the screening platforms are applicable to other ingredient types. Fractions identified through these screens will subsequently be evaluated for host-modulating effects, building toward a mechanistic understanding of how bioactive feed ingredients interact with gut health.

Antioxidant Supplementation in Maternal Diet Modulates the Effects of Season on Swine Progeny

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The prenatal environment plays a vital role in shaping postnatal development. Maternal heat stress can impair foetal growth and result in intrauterine growth restriction (IUGR), particularly under production conditions that pose thermoregulatory challenges, such as the outdoor farming of Iberian pig sows. Dietary strategies aimed to alleviate the detrimental effects of heat stress (e.g. antioxidant supplementation) could benefit traditional outdoor Iberian pig rearing. This study intended to evaluate the effects on growth and the development of head and brain, as sign of IUGR, of two maternal antioxidant strategies and their interaction with gestational season. Two trials were carried out: one under thermoneutral conditions (TN; gestation from November to March) and other under heat stress conditions (HS, gestation from May to September). Seventy-two Iberian sows per season were allocated to three dietary groups (n = 24) from day 50 of gestation until weaning: a) C (control), 100 IU vit E/kg and inorganic sources of minerals (Mn, Zn, Cu, Se) according to FEDNA 2013; b) VE, supplemented with 200 IU vit E/kg; and c) OM, including 45%–100% organic trace minerals to enhance bioavailability. Piglets were weighted at 0, 15 and 21 days (d). At birth, piglets (n=699) were classified as small (S), medium (M) or large (L) according to birthweight (BiW). At 21 d, 108 male piglets (48 born under HS and 60 under TN, half S and half L in each season) were measured and weighted. Then, they were euthanised to obtain brain and head weights and measures. Data were analysed using a linear model including the diet, BiW and season and their interaction and a Tukey post hoc test performed in R 4.5.1. Significance was considered with $p < 0.05$. At birth there were no significant differences between diets nor seasons. However, at 15 and 21 d, piglets born under HS were lighter and smaller than those born under TN. At 21 d, a season*BiW interaction was observed: L and M piglets were heavier in winter than summer, but S piglets had the same weight regardless of season. At this age, OM piglets were heavier than C, whereas no differences were found between VE with OM or C. S piglets had heavier brains and heads and higher brain/body weight ratio than L offspring. Neurodevelopmental traits appeared more sensitive to maternal antioxidant supplementation under HS conditions. Thus, OM offspring born under HS showed heavier heads and brains than C or VE piglets, while no significant differences were observed in TN conditions. However, the brain-to-head weight ratio was higher in VE offspring under HS compared to TN conditions, with no season variations in C nor OM groups. Relative head size showed a diet×season interaction: it was larger in C and VE litters born under HS than TN conditions, whereas OM offspring showed similar values across seasons. Results show that effects on the progeny of the maternal antioxidant supplementation were modulated by external conditions. Under heat stress, OM diet was associated with improved body development, reducing signals of IUGR related to head and brain size. In conclusion, these findings highlight the importance of season-specific nutritional strategies to optimize offspring development in Iberian pig production systems This work was financially supported by PID2022-139367OB-C22 and PREP2022-000441 funded by MICIU/AEI/10.13039/501100011033 (FEDER/UE, FSE+).

Immunomodulatory effects of nucleotide-based formulations enriched with seaweed and olive bioactive compounds in mammals: evidence from an LPS-induced rat model

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Background and objective: Nutritional immunomodulation is a One Health-aligned strategy to enhance host resilience and reduce reliance on antimicrobials, thereby contributing to antimicrobial resistance mitigation. Bioactive compounds derived from seaweed, olive tree, and nucleotide-rich yeast extracts have demonstrated immunomodulatory properties and synergistic activity in previous invertebrate models, supporting their potential as feed-based immunomodulatory alternatives. This study aimed to evaluate these compounds in a preclinical lipopolysaccharide (LPS)-induced inflammatory/infectious-like rat model to assess their potential health benefits for livestock and companion animals. Methods: Fifty 8-week-old male Wistar rats were allocated to five groups (n = 10) and orally supplemented for 42 days with either nucleotides (NU; Nucleoforce® HF), nucleotide formulations enriched with seaweed meal (NUS; Nucleoforce® Immunity), or olive extract (NUO; Nucleoforce® Performance) at 107 mg/kg, or with vehicle alone (1 mL/kg condensed milk diluted 1:4 with water; positive and negative control groups). All products were supplied by Bioiberica SAU (Spain). Rats received an intraperitoneal challenge of 500 mg/kg LPS on day 36, followed by 300 mg/kg LPS daily from day 37 to day 42, except for the negative control group, which received saline injections from day 36 to day 42. Morphometric parameters, hematological profiles, gene expression in spleen and peripheral blood mononuclear cells (PBMCs), biochemical parameters in blood and small intestine, and histological analyses of ileum and colon were evaluated. Results: All supplemented groups demonstrated significant immunomodulatory effects by mitigating LPS-induced damage. The nucleotide-based formulations showed specific effects on the expression of genes associated with inflammation and Th1/Th17 immune responses in PBMCs (including Rorc, IL-10, Tnf, and Tbx21) as well as in spleen tissue (including Cd4, Cd14, Cd19, and S100a9). Furthermore, the NUS and NUO formulations significantly reduced circulating IL-10 levels and improved hematological parameters, including red blood cell count, hematocrit, and hemoglobin concentration. No significant effects on tissue histology were observed. Discussion & conclusion: The nucleotide-based formulations evaluated in this study demonstrated clear immunomodulatory potential and may represent effective nutritional tools to enhance health and performance in livestock and companion animals, particularly under conditions associated with anemia or immunosuppression. The previously described synergistic interactions among these bioactive compounds of diverse origin were reflected in the coordinated regulation of hematological, transcriptional, and inflammatory markers in the study herein. Overall, supplementation positively influenced immune balance across Th1, Th2, Th17, and Treg axes, as well as B cell-related signaling, together with hematological status and systemic resilience. These findings support the use of such formulations as sustainable nutritional strategies to reduce dependence on antimicrobials and growth promoters in different animal species.

Anti-inflammatory effect of Cyanidin-3-O-lathyroside from Eleutherococcus sessiliflorus in LPS-induced in vitro model

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Natural bioactive compounds derived from plants have attracted increasing attention as potential feed additives for improving immune health in livestock. Cyanidin-3-O-lathyroside, an anthocyanin compound found in various plant sources, has been suggested to possess anti-inflammatory properties; however, its immunomodulatory mechanisms remain poorly understood. This study investigated the anti-inflammatory effects of cyanidin-3-O-lathyroside in lipopolysaccharide (LPS)-stimulated RAW 264.7 macrophages, a widely used model for studying innate immune responses. Macrophage activation was assessed by examining cell morphology and quantifying pro-inflammatory cytokine expression using quantitative PCR (qPCR). Extracellular cytokine levels and inflammatory mediator activity were measured using ELISA and nitric oxide (NO) assays. The underlying molecular mechanism was further examined through immunoblot analysis of nuclear factor-kappa B (NF-κB) signaling pathway targets and immunofluorescent staining of p65 nuclear translocation. Treatment with cyanidin-3-O-lathyroside significantly attenuated macrophage activation and reduced both mRNA and protein expression levels of pro-inflammatory cytokines, including tumor necrosis factor alpha (TNF-α), interleukin-1 beta (IL-1β), and interleukin-6 (IL-6). In addition, the expression of inflammatory mediators such as inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) was markedly decreased. Mechanistically, cyanidin-3-O-lathyroside inhibited the phosphorylation of IκB-α and suppressed the nuclear translocation of NF-κB p65 in a dose-dependent manner. These findings demonstrate that cyanidin-3-O-lathyroside effectively suppresses LPS-induced inflammatory responses through modulation of the NF-κB signaling pathway. The results suggest that this compound may have potential as a natural anti-inflammatory bioactive compound for regulating immune responses in livestock, supporting its possible application as a functional feed additive to improve animal health and resilience.

Effects of a phytogenic blend of capsicum, clove and garlic in starter feed on the growth performance of Holstein dairy calves.

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A phytogenic blend of capsicum oleoresin, clove essential oil and garlic essential oil (Fytera™ Advance) has demonstrated a positive impact on growth performance metrics in livestock species yet has not been examined in dairy calves. The objective was to investigate the effects of this phytogenic blend, supplemented in calf starter, on the growth performance of Holstein dairy calves from birth to 15 weeks of age. The study took place at a commercial calf-rearing facility in Ontario, Canada from October 2024 to June 2025. A total of 283 Holstein dairy calves (136 heifers, 147 bulls) were enrolled in one of two dietary treatments: 1) a negative control of textured starter feed (CON) and 2) the same starter feed with 375 g/MT of the phytogenic blend (FA). Calves were individually housed from birth to weaning and bottle-fed 9 L/d of milk replacer (3 feeds × 3 L), until a two-step weaning process between weeks 8 and 11. Starter feed was offered ad libitum from the start of trial, with individual intakes recorded until the end of the weaning phase at 11 weeks. Calves were then transferred to group-housing, maintaining groups of the same dietary treatments, yet intakes were no longer recorded. Individual BW were recorded at birth, 7, 11 and 15 weeks. Visual health scoring of eye and nasal discharge, ear position and faecal consistency on a 4-point scale (from 0-3, least to most severe) were recorded and treatment records were collected. Calculated measures included ADG, and feed efficiency (FCR and F:G). Analyses were completed in SAS Studio. Cumulative intakes and scour score frequency and severity were analyzed in PROC MIXED, with fixed effects of treatment. PROC GLIMMIX was utilized for BW, ADG, FCR and F:G with fixed effects of treatment, week, and their interaction. Sex and birthweight category (≤ 36 kg = Low, 37- 46 kg = Mid, and > 46 kg = High) were included as covariates in all models. Calf within treatment was a random effect, with the repeated measure of week. Body weight, FCR and F:G utilized a lognormal distribution and identity link, with reported values back-transformed to the data scale. A Gaussian distribution with identity link was utilized for ADG. Calves on FA had a tendency for greater mean BW compared with CON (P=0.066), with FA calves having a 4.4 kg greater mean BW at 15 weeks. No significant difference was detected in the ADG of FA calves compared to the CON (P = 0.427). There were no significant differences in cumulative intakes between treatments (P = 0.771). However, there was a tendency for greater starter intakes among the FA calves compared to the CON calves during the pre-weaning phase from birth to week 7 (CON = 7.8 kg ± 0.44 SE, FA = 8.8 kg ± 0.46 SE; P = 0.088). No significant differences in feed efficiency (FCR, P = 0.946; F:G, P = 0.639) were detected. Less than 1% of eye, ear and nasal scores, and 6% of faecal scores were ≥1. There tended to be a higher proportion of CON calves receiving at least one treatment for scours (CON = 70%, FA = 59%; P = 0.053). However, a low median age of 10-12 d at first treatment when calves had consumed < 200 g of starter on average suggests this difference may be due to a proactive farm treatment protocol for scours. These findings suggest this phytogenic blend may be a promising calf starter additive and further investigation of the health impacts of FA under a variety of commercial farm conditions is warranted.

Natural bioactives preserve gut resistome stability in broilers compared with antibiotic growth promoters

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The increasing concern over antimicrobial resistance in livestock production highlights the need for sustainable alternatives to antibiotic growth promoters with nutribiotic potential. Two hundred broiler chickens were allocated to four dietary treatments with five replicates each: Control [CON; 100% of the recommended synthetic vitamin E (80 IU VitE/kg)], ANTIB (100% VitE plus 55 mg/kg zinc bacitracin), CW10, and CW0. CW10 and CW0 diets contained 10 and 0 IU VitE/kg feed, respectively, and were supplemented with 100 mg gallic acid equivalents of polyphenols from grape pomace, 150 mg β-glucans from wine lees, and 25 mg canthaxanthin equivalents from mandarin cold-pressed essential oil per kg feed. Cecal digesta collected at 35 days were analyzed using shotgun metagenomics and metatranscriptomics to characterize the gut resistome and transcriptional activity of antibiotic resistance genes (ARGs). Resistome richness and total ARG abundance did not differ among treatments. Antibiotic supplementation produced selective shifts in resistance-related functional categories and increased signals linked to microbial stress adaptation and communication. In contrast, bioactive diets showed resistome profiles comparable to the control and did not enrich resistance-associated genes. Metatranscriptomic analysis showed that antibiotic supplementation activated ARG classes associated with macrolide–lincosamide–streptogramin, tetracycline, fluoroquinolone, nitroimidazole, and phenicol resistance. This was accompanied by increased expression of DevR, capsular polysaccharide synthesis protein Cap8G, rhamnan synthesis proteins, glycosyltransferase WbuB, and polysaccharide biosynthesis tyrosine autokinase, along with higher expression of polyphosphate kinase and β-lactamase induction pathways. In contrast, CW10 and CW0 maintained ARG expression comparable to or lower than the control, with the only notable shift being increased expression of the exopolysaccharide biosynthesis glycosyltransferase VpsI. Overall, the bioactive mixtures did not promote resistome expansion or ARG activation, supporting their potential as nutribiotic alternatives to growth promoters in broiler production.

Evaluation of thinned apple by-products in ETEC F4-challenged weaned piglets

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Post-weaning diarrhea induced by enterotoxigenic Escherichia coli (ETEC) F4 represents a major cause of economic losses and antimicrobial use in pig production. Thinned apples (TA), an underutilized by-product of apple orchards rich in polyphenols and other fermentable substrates, may offer a nutritional approach to support gut health during this critical period. This study evaluated the effects of dietary TA supplementation on growth performance, fecal consistency and selected blood parameters in ETEC F4-challenged piglets. At weaning (d-4), 90 ETEC F4-susceptible piglets (25 ± 3 d of age) were allocated to three post-weaning diets (10 pens/treatment; 3 piglets/pen): a standard starter diet (CON), or isonitrogenous and isoenergetic diets supplemented with 3% (TA3) or 6% (TA6) TA . Four days after weaning (d0), piglets were orally challenged with 2 mL of ETEC F4 (10^{1}□ CFU/mL). Body weight (BW) was recorded at d−4, d0, and on days 3 and 9 post-infection. Average daily gain (ADG) and feed intake were calculated for defined intervals. Fecal score was recorded daily, and days with diarrhea (score ≥ 3) were determined. Data were analyzed using linear mixed models with diet as fixed effect and run and pen as random effects. At weaning, piglets had similar BW (7.60 ± 0.85 kg; P > 0.10), and no dietary effects on BW were detected at any subsequent time point (P > 0.10), reaching a simiar (P > 0.10) final BW of 8.95 ± 0.563 kg at the end of the trial. The ADG and feed intake were not affected by TA inclusion (P > 0.05). However, a tendency (P = 0.05) for differences in ADG was observed in the 0–3 day period, where the lowest value was observed in TA3 pigs (0.011 ± 0.005 kg/d) compared to TA6 (0.024 ± 0.012 kg/d) and CON (0.039 ± 0.019 kg/d). Regardless of the treatment, the fecal score significantly changed over time (P < 0.001), with most of the piglets developed diarrhea with the maximum occurrence being between 2 (fecal score 3.06 ± 0.25) and 4 (fecal score = 3.07 ± 0.21) days post-infection. No severe diarrhea (fecal score = 4) were observed. Fecal score and the percentage of piglets with diarrhea were comparable (P > 0.10) between the groups along all the different time points investigated. The days in diarrhea in CON (4.73 ± 2.49 days), TA3 (4.63 ± 2.78 days) and TA6 (4.83 ± 2.93 days) were also similar (P > 0.10) between the groups. Serum biochemical parameters (creatinine, urea, non-esterified fatty acids and glucose) were not influenced (P > 0.10) by the treatment. In conclusion, inclusion of 3% or 6% TA did not improve growth performance or affect clinical signs to ETEC F4 challenge.

Supplementation of a porcine-derived bioactive complex in the diet reduces post-weaning diarrhea and modulates gut microbiota in ETEC-challenged piglets

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Post-weaning enteric dysfunction impairs pig health and performance. This study evaluated the effects of increasing dietary levels of a porcine-derived bioactive complex (CBS) containing mucosal-associated components (mucin, secretory IgA, phospholipids, sphingolipids, lysozyme and intestinal alkaline phosphatase), as an alternative to antibiotic growth promoters, on the growth performance, diarrhea incidence (DI), and gut microbiota in piglets weaned at 21 or 25 days of age (WA) and challenged with Escherichia coli (ETEC) F4+ K88. A total of 144 piglets were randomly assigned to a 5 × 2 factorial design, with 5 treatments and 2 WA (21 and 25 d). Tested treatments consisted of: T1 (basal diet; negative control), T2 (basal diet + 120 mg/kg chlorohydroxyquinoline), and T3-T5 (basal diet + CBS at 830, 2,000, or 4,100 U/kg, respectively). Piglets were orally challenged with ETEC F4 on day 14, and the trial lasted 35 days. Additionally, a non-challenged group fed the basal diet (T6) was included at 21 and 25 d of age. Average daily gain (ADG), average daily feed intake (ADFI), feed conversion ratio (FCR), and DI were analyzed separately for the pre-challenge (d 1–14) and post-challenge (d 15–35) periods using ANCOVA models including treatment, WA, and their interaction as fixed effects, with initial body weight as a covariate. Treatment dose-response was also evaluated by modeling treatment level as a quantitative variable. When appropriate, multiple comparisons were performed using Dunnett's test with T1 or T2 as control groups. An additional model including all treatments was used to compare challenged groups (T1–T5) with the non-challenged group (T6). On d 35, 48 piglets were euthanized for microbiota composition and characterized by gene sequencing. Alpha and Beta diversity, and differential taxonomic abundance (LEfSe) were subsequently evaluated. During d 1-14, ADG, ADFI, and FCR did not differ among treatments ($P > 0.05$), whereas piglets weaned at 25 d showed greater ADG than those weaned at 21 d ($P < 0.001$). After the challenge, WA affected final body weight ($P = 0.0038$) and ADG ($P = 0.0120$), but treatments did not influence ADG, ADFI, or FCR ($P > 0.05$). A dose-dependent tendency for reduced diarrhea incidence was observed with increasing CBS levels in both periods (pre: $P = 0.083$; post: $P = 0.0949$). In multiple comparisons, T5 showed lower diarrhea incidence than T2 (pre: $P = 0.046$; post: $P = 0.0499$). No differences were detected between challenged treatments (T1–T5) and the non-challenged (T6) for any variable ($P > 0.05$). Microbiome analysis revealed high Escherichia abundance across challenged groups, consistent with the ETEC F4+ infection. Alpha diversity did not differ ($P > 0.05$), but Beta diversity showed greater dispersion in T1, whereas T4 clustered more tightly, indicating greater homogeneity in microbiota response within that treatment. LEfSe identified Streptococcus enriched in T1 and reduced in higher CBS groups, especially T4/T5 ($P < 0.05$). Overall, dietary supplementation with the CBS did not alter growth performance of ETEC-challenged piglets, while WA remained an important determinant of post-weaning growth. Nonetheless, increasing CBS levels mitigated diarrhea incidence and stabilized gut microbial composition, as evidenced by lower Streptococcus abundance and improved microbiota homogeneity. These findings suggest that CBS could serve as a viable alternative to antibiotic growth promoters during post-weaning enteric challenges.

Improving Zootechnical Performance of Broilers with Polyphenol Blend

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The poultry industry prioritizes rapid growth, feed efficiency, and breast yield through intensive breeding and management. While these improvements have advanced production, they have also led to challenges with meat quality and raised concerns about animal welfare and performance. This study investigated how a polyphenol blend (PB) with strong antioxidant properties influences broiler performance, carcass traits, and incidence of myopathies. A total of 760 male Ross chicken was distributed in two groups (control and 300g/ton of PB) in a randomized experimental design, with ten replicates of 38 birds each. The parameters evaluated: weekly zootechnical performance, carcass yield and scores of myopathies (at 42 days). By day 42 PB group had significantly higher body weight (BW) (3668.5 g) and cumulative weight gain (WG) (3623.4 g) compared to control BW (3565.4 g) and WG (3520.4 g) ($p \leq 0,05$). Feed conversion ratio (FCR) was significantly improved in PB group (1.506 versus 1.556 ($p \leq 0,05$)), while feed intake (FI) remained consistent across both groups, indicating that performance improvements resulted from better efficiency. The final weight of the birds selected for carcass yield and cuts with a variation of ±5% from the group averages was significantly higher for PB group (3.480 kg vs. 3.400 kg; $p \leq 0,05$), with significantly higher weight of legs (0.832 g) compared to control group with weight of legs (0.799g). Wings, breast, boneless breast, back and feet are not significant. Carcass and cuts yield (%) did not differ significantly. PB group showed numerical, though non-significant, reduction in severe wooden breast incidence with 54% lower risk of having more severe WB (10,3% of severe WB score vs 27,6% of severe WB score in control group). A slight reduction in incidence of white striping was observed at 42 days for PB group compared to control (1.45 vs 1.47). In summary, these findings indicate that polyphenol blend with high antioxidant activity can improve performance and carcass yield of broilers under intensive breeding system.

Assessing accuracy and on-farm applicability of dairy cow feed intake prediction equations

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Precise dry matter intake (DMI) estimation is essential for performance-adapted ration formulation in dairy cows. This study evaluated the accuracy and applicability of 15 established DMI equations using data from Austrian research trials. The dataset comprised 76 dairy cows (Holstein-Friesian, Fleckvieh) across lactation (DIM 20–300 d), parities (1–4), body weights (550–750 kg), milk yields (20–55 kg/d), and concentrate intakes (4–10 kg DM/d). Diets reflected regional practice: grass silage (35–45% NDF DM), maize silage, and concentrates, resulting in total DMI of 15–28 kg/d. Actual DMI was calculated as offered minus refused feed (<5% refusals). Fifteen models were tested: simple (2–3 variables: Vadiveloo & Holmes 1979; Yungblut et al. 1980; Rayburn & Fox 1993), moderate (4–6: NRC 2001; Lee et al. 2021; Tylutki et al. 2008; Freitas et al. 2006; Santos et al. 2009; Souza et al. 2014; Fuentes Pila et al. 2003; Jeroch et al. 2008), and complex (≥7: Allen et al. 2019; Gruber et al. 2004, formulas 1 and 6; Chase & Sniffen 1985). Inputs included BW^0.75, milk yield (MY), fat yield (FY), DIM, forage and ration NDF (FNDF, RNDF), and energy density (MJ NEL/kg DM). Outliers (>2.5 SD) were removed, <5% missing values imputed, redundancies eliminated, and data aggregated to daily means. Accuracy metrics were sum of squared errors of prediction (SSEP; primary), mean bias (predicted–observed), R^2, and ANOVA ($p<0.05$). Bland–Altman plots assessed bias; applicability was rated by variable count (easy ≤5; moderate 6–7; complex >7). Gruber et al. (2004) Formula 1 showed lowest SSEP (R^2=0.92, bias −0.12 kg/d), followed by Formula 6 (bias +0.08 kg/d) and Tylutki et al. (2008) (R^2=0.89, bias −0.21 kg/d). NRC (2001) ranked mid-tier; Allen et al. (2019) underperformed, likely due to unmodeled parity and DIM effects. Seven models (e.g., Tylutki, NRC, Vadiveloo) were easily applicable using routine BW, MY, and FY data. Bland–Altman plots indicated minimal bias at 18–22 kg/d DMI, with overprediction in early lactation and underprediction at peaks (>25 kg/d). Predictions were tighter for Fleckvieh than Holstein-Friesian. ANOVA revealed significant differences ($p<0.01$), with the top three models outperforming others by 15–25% SSEP. Tylutki et al. (2008) offered the best balance of accuracy and practicality for Central European farms, while Gruber models suit research with full datasets. These equations support precise feeding, reducing energy deficits (ketosis risk), excesses (overconditioning, fertility loss), feed costs, and N/P excretion. Regional validation highlights the need for models tailored to silage-based, moderate-yield systems. Integration into management software could increase milk yield by 2–5% and reduce feed waste by 5–10%, advancing sustainable dairy nutrition. Literature Allen et al., J Dairy Sci 2019;102:2682–2696 Chase & Sniffen, J Dairy Sci 1985;68:871–879 Freitas et al., J Dairy Sci 2006;89:3102–3112 Fuentes Pila et al., J Dairy Sci 2003;86:111–118 Gruber et al., J Anim Physiol Anim Nutr 2004;88:251–263 Jeroch et al., Tierernährung 2008;36:45–67 Lee et al., J Dairy Sci 2021;104:8285–8298 NRC, J Anim Sci 2001;79:249–263 Rayburn & Fox, J Dairy Sci 1993;76:1411–1418 Santos et al., J Dairy Sci 2009;92:3437–3446 Souza et al., J Dairy Sci 2014;97:7499–7509 Tylutki et al., J Dairy Sci 2008;91:1945–1954 Vadiveloo & Holmes, J Dairy Sci 1979;62:1425–1429 Yungblut et al., Can J Anim Sci 1980;60:601–609

Accuracy of five dry matter intake prediction models in lactating dairy cows across energy-corrected milk yield classes: a meta-analysis

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Accurate prediction of dry matter intake (DMI) is fundamental to ration formulation and precision feeding in dairy production. Five mechanistic models are applied to estimate DMI in lactating dairy cows: NRC (2001), NASEM (2021), the Cornell Net Carbohydrate and Protein System (CNCPS), GfE (2023), and Agroscope; however, their comparative accuracy across different production levels has not been systematically evaluated in a large meta-analytic framework. We hypothesised that predictive performance would differ significantly among models and would be modulated by the cow's energy-corrected milk (ECM) yield class. A database of 429 treatment means was assembled from 132 peer-reviewed studies published between 1984 and 2025, comprising 6,827 Holstein cows housed exclusively in freestall systems and fed total mixed (TMR) or partial mixed (PMR) rations. Only studies using completely randomised (CRD) or randomised block (RBD) designs were included; Latin square and crossover experiments were excluded. Treatment means were classified into three ECM yield classes: Class 1 (<30 kg/d; n=113; mean=24.7 kg/d), Class 2 (30–40 kg/d; n=174; mean=35.1 kg/d), and Class 3 (>40 kg/d; n=142; mean=46.1 kg/d). Prediction accuracy was assessed using mean prediction bias, root mean square error (RMSE), and Lin's concordance correlation coefficient (CCC). Overall, NASEM (2021) yielded the lowest RMSE (2.41 kg/d; bias=+0.11 kg/d; CCC=0.735) and NRC (2001) achieved the highest CCC (0.781; bias=−0.48 kg/d; RMSE=2.68 kg/d). Agroscope showed moderate accuracy (bias=−0.66 kg/d; RMSE=2.60 kg/d; CCC=0.690), while GfE (2023) showed the greatest systematic underprediction (bias=−1.96 kg/d; RMSE=2.87 kg/d; CCC=0.683) and CNCPS underpredicted consistently across all classes (bias=−1.60 kg/d; RMSE=2.71 kg/d; CCC=0.729). In Class 1 cows, NRC (2001) showed the lowest RMSE (2.45 kg/d) and highest CCC (0.588), while NASEM (2021) had the smallest bias (+0.27 kg/d) but the lowest CCC observed across all classes (0.475). GfE (2023) performed worst in this class (bias=−2.45 kg/d; RMSE=3.35 kg/d; CCC=0.355), followed by CNCPS (bias=−1.91 kg/d) and Agroscope (bias=−1.06 kg/d). In Class 2, NASEM (2021) yielded the lowest RMSE (2.23 kg/d; bias=−0.03 kg/d) and NRC (2001) the highest CCC (0.744; bias=−0.29 kg/d), whereas Agroscope (bias=−1.04 kg/d), CNCPS (bias=−1.84 kg/d), and GfE (bias=−1.92 kg/d) continued to underpredict. In Class 3 cows, CNCPS showed the lowest RMSE (2.46 kg/d; CCC=0.747) despite persistent underprediction (bias=−1.05 kg/d), and NRC (2001) achieved the highest CCC (0.753) but the highest RMSE (3.19 kg/d; bias=−0.72 kg/d). NASEM (2021) offered the most balanced performance in this class (bias=+0.14 kg/d; RMSE=2.57 kg/d; CCC=0.688), while Agroscope showed near-zero bias (+0.12 kg/d) but poor agreement (CCC=0.533; RMSE=2.78 kg/d). No current model provided adequate DMI predictions across all ECM classes. NASEM (2021) is recommended as the most balanced model overall, given its consistently low RMSE and near-zero bias. GfE (2023) requires revision across all production levels; CNCPS (2015) requires improvement particularly in Classes 1 and 2, although its low RMSE in Class 3 suggests some sensitivity to high-production conditions. Development of ECM class-specific equations would strengthen precision feeding tools and support resource efficiency in circular dairy systems.

Use of real-time forage-harvester NIRS to reduce whole-plant maize silage nutrient variability through dynamic cutting-height control

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Cutting height is a tool to modify whole-plant maize silage composition, but it typically involves a trade-off between fibre concentration and DM yield. Increasing cutting height reduces DM yield while decreasing NDF (Diepersloot et al., 2026; Aiello et al., 2008). However, fixed cutting-height settings do not account for within-field variability, which can increase silage heterogeneity and limit precision feeding. We hypothesised that a NIRS–guided dynamic cutting-height approach can increase the proportion of harvested material within a target NDF range and reduce within-field variability, while limiting DM yield losses compared with fixed settings. The aim was to regulate the level and variability of nutritional components during whole-plant maize silage harvest by dynamically adjusting cutting height using on-board NIRS. An on-farm trial was conducted on whole-plant maize (Don Mario 2789) at a dairy farm in Villa María, Córdoba, Argentina, in collaboration with John Deere Argentina. Harvest was performed using a John Deere 8500i self-propelled forage harvester equipped with HarvestLab 3000 NIRS sensors. Treatments were fixed cutting height 15 cm (H15), fixed 45 cm (H45), and variable cutting height (HVar; switching among 15, 25 or 45 cm). In HVar, header height was adjusted to maintain 33–38% NDF based on real-time readings (if NDF >38% for ≥5s, raise header; if NDF <33% for ≥5s, lower header). The design was a randomised complete block with five strip-plot replicates per treatment (7.5 m×~200 m), yielding ~520 observations per replicate. Treatment effects were tested by ANOVA (Fisher's LSD, $p<0.05$). Dispersion was assessed with Levene's test, SD and CV. For a practical interpretation, useful DM (tDM/ha) within the target range was calculated as yield × in-range fraction, and the land requirement to supply 3.1 tDM/cow/year was estimated as ha/cow/year= 3.1/useful DM. DM yield differed among treatments ($p<0.05$): H15= 15.36±3.42 tDM/ha; HVar= 13.82±3.40 tDM/ha; H45= 12.32±3.79 tDM/ha. NDF also differed ($p<0.05$): H15= 37.22±1.90%; HVar= 35.23±1.67%; H45= 34.37±1.91%. NDF variability (CV) was lowest in HVar (4.73%) vs H15 (5.11%) and H45 (5.56%), with differences in dispersion ($p<0.01$). The proportion of readings within 33–38% NDF was 65.0%, 73.3% and 86.6% for H15, H45 and HVar, respectively. The mean DM yield associated with this in-range dataset was 14.9, 12.3 and 13.8 tDM/ha for H15, H45 and HVar, respectively. Consequently, the estimated land requirement to supply target-quality DM was 0.32, 0.34 and 0.26 ha/cow/year for H15, H45 and HVar, respectively. Consistent with published evidence, increasing cutting height resulted in relative decreases in DM yield and NDF. DM yield showed a relative decrease of 6.60% per 10 cm increase (from 15 to 45 cm), which is larger than the relative decreases reported by Diepersloot et al.(2026) (~3.80% per 10 cm) and Aiello et al.(2008) (~3.70% per 10 cm). Likewise, NDF showed a relative decrease of 2.55% per 10 cm in this study, compared with ~2.40% in Diepersloot et al.(2026) and 2.2-3.5% in Aiello et al.(2008). In this context, the dynamic strategy reduced within-field variability (lower CV) and increased the fraction of harvested forage within the nutritionally defined NDF window (33–38%), thereby improving maize silage uniformity while avoiding the larger DM yield losses associated with a high fixed cutting height. Overall, these findings support the hypothesis that real-time NIRS–guided cutting-height control can help deliver more uniform maize silage and provide a practical pathway to tighter diet formulation in precision feeding systems.

Artificial intelligence applied to feed delivery in dairy farms

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Feeding management represents one of the major cost components and a key factor influencing productivity in dairy production systems. Despite the increasing adoption of precision livestock farming technologies, daily feed allocation adjustments in dairy herds are still commonly based on heuristic rules and manual observations, which limits the ability to adapt to dynamic variations in animal intake and environmental conditions. In this context, this study proposes an automatic recommendation model for daily feed allocation in dairy herds based on machine learning techniques. The study was conducted on a commercial Brazilian dairy farm with 294 lactating cows, integrating multiple sources of zootechnical, nutritional, climatic, and herd management data. Three supervised regression models — Linear Regression, Random Forest, and XGBoost — were evaluated to predict next-day total mixed ration intake for different groups of cows. The dataset comprised 1,087 days of data collection and 5,404 feed preparation records, including variables such as milk production, herd size, amount of feed offered, feed refusals, days in milk, and climatic conditions. The models were trained using three-fold cross-validation and evaluated using the coefficient of determination (R2) and Mean Absolute Error (MAE). The results showed that the Linear Regression model achieved the best predictive performance, reaching R2 = 0.88 and MAE = 96.4 kg, demonstrating a strong ability to capture variations in feed intake across different cow groups. Furthermore, the application of the model enabled a reduction in feed waste during the test period, highlighting its potential as a decision-support tool for feed management and for improving the economic efficiency of dairy operations. Overall, the results indicate that the integration of heterogeneous data sources combined with machine learning models represents a promising approach for advancing precision livestock farming and developing intelligent decision-support systems for dairy farms.

A pilot study integrating jaw accelerometry and surface electromyography for automated monitoring of chewing dynamics in cattle

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Precision livestock farming increasingly relies on sensor-based monitoring systems to quantify feeding behaviour and support more efficient and sustainable feed management. Mastication dynamics play a key role in feed particle breakdown, saliva production, rumen buffering, and ultimately feed utilization efficiency. However, objective quantification of chewing mechanics and associated muscular effort remains challenging under practical conditions, particularly when attempting to synchronise heterogeneous sensor data streams and capture both jaw kinematics and muscle activity simultaneously. This pilot study investigated whether integrating multimodal sensors combining jaw-mounted accelerometers with surface electromyography (sEMG) could enable automated detection of chewing cycles and provide quantitative indicators of mastication dynamics in cattle. Jaw movements were recorded using upper and lower jaw triaxial accelerometers (AX3, Axivity Ltd., Newcastle upon Tyne, UK; sampling frequency 100 Hz), while bilateral masseter muscle activity was measured using wireless sEMG sensors with integrated inertial channels (Trigno™, Delsys Inc., Natick, MA, USA; EMG sampling frequency 2000 Hz). Two heifers were monitored repeatedly across thirteen feeding sessions in a cross-over design including hay and pellet diets. Chewing bouts were detected from lower-jaw acceleration signals and individual chewing cycles were identified using peak-detection algorithms. sEMG envelopes were synchronised with accelerometer signals using cross-correlation–based lag estimation to compensate for asynchronous sensor start times. For each bout, chewing frequency, a grinding amplitude proxy derived from the median absolute deviation (MAD) of the chew-band filtered difference between lower and upper jaw accelerometers, and cycle-level muscular effort (median integrated sEMG area per cycle) were calculated. Delsys inertial channels (masseter and forehead) were explored as an internal reference to support robustness against jaw-sensor disturbances. A total of 25 chewing bouts were successfully aligned between accelerometer and sEMG recordings. Chewing frequency ranged from 1.31 to 1.73 Hz across feeding conditions. Median chewing frequency was 1.49 Hz (IQR 1.47–1.56) for hay and 1.46 Hz (IQR 1.41–1.58) for pellet feeding. Grinding amplitude (MAD) tended to be higher during hay feeding than pellet feeding, indicating greater jaw excursion during fibrous feed processing. When considered jointly with chewing frequency, hay bouts generally occupied a region characterised by higher grinding amplitude, suggesting increased mechanical processing during fibrous feed consumption. These results demonstrate the feasibility of combining accelerometry and sEMG for automated quantification of chewing behaviour and muscular effort in cattle. Such multimodal sensor approaches provide a methodological framework for characterising mastication dynamics and may support future precision feeding research by linking chewing patterns with feed physical properties, intake behaviour, and digestive efficiency. In future applications, data derived from this approach could contribute to predictive models relating mastication dynamics to feed processing and utilization across diets, breeds, and age groups, supporting the development of sensor-based indicators of feed processing and rumination efficiency.

Comparative assessment of 3-dimensional camera and GreenFeed systems for on-farm feed efficiency evaluation

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Improving feed efficiency (FE) in dairy cattle can reduce greenhouse gas emissions per unit of milk and enhance profitability. Routine genetic improvement, however, is hindered by the high cost of measuring dry matter intake (DMI). Technologies such as the GreenFeed system (C-Lock Inc.), which quantifies gas exchange, and 3D camera–based system for estimating DMI may offer practical solutions for large-scale on-farm phenotyping. This study aimed to: 1) compare cow rankings for 3D camera-derived DMI (3D_DMI) with rankings for GreenFeed-derived gas exchange and efficiency traits, and 2) evaluate the relationship between 3D_DMI and DMI predicted from energy-corrected milk (ECM) and gas exchange using published equations. Data were collected from October 2024 to October 2025 on a 250-cow commercial dairy farm (Maitoaho, Kiuruvesi, Finland) equipped with both technologies. A subset of 112 cows was monitored while receiving a grass silage–based total mixed ration (forage-to-concentrate ratio 51:49, DM basis). The 3D camera provided individual DMI estimates, while the GreenFeed system recorded daily methane (CH_4), carbon dioxide (CO_2), and oxygen (O_2) exchange, from which heat production (HP) was calculated. Cow-level least-squares means for 3D_DMI, ECM, and GreenFeed-derived traits (CH_4, CO_2, O_2, HP, ECM/HP, and CH_4/ECM) were estimated by parity using PROC MIXED in SAS, with a cow × parity fixed effect and cow within parity as a repeated measure. Based on these means, cows were ranked separately for each trait (rank 1 = optimal for the respective trait). Agreement between ranking methods was evaluated using Spearman rank correlations (ρ), reranking magnitude, and ranking consistency within the top 25% of cows. Dry matter intake predicted from ECM and gas-based equations were regressed on 3D_DMI using PROC MIXED (random cow within parity effect). Model performance was assessed by coefficient of determination (R^2), root mean square prediction error (RMSPE), concordance correlation coefficient (CCC), and mean square error decomposition. The ECM followed a typical lactation pattern, peaking at about 39 kg/d around week 7 in lactation before gradually declining to about 30 kg/d by late lactation. The camera-derived DMI increased from 19 kg/d in early lactation to about 25 kg/d in late lactation. Rank correlations between 3D_DMI and GreenFeed-derived traits were weak to moderate (−0.23 to 0.34). The strongest associations were observed for O_2 ($\rho = 0.34$), HP ($\rho = 0.32$), and CH_4 ($\rho = 0.30$), followed by CH_4/ECM and ECM/HP ($|\rho| = 0.23$), and CO_2 ($\rho = 0.21$). Energy corrected milk showed essentially no association with 3D_DMI ranking ($\rho = -0.04$). Substantial reranking occurred across traits, with mean shifts of 28–41 positions and only 21–50% ranking consistency among the top 25%-ranked animals. Regression analyses showed weak relationships between 3D_DMI and DMI predicted from ECM and gas-based equations. Across models, R^2 ranged from 0.03 to 0.20 and CCC from 0.05 to 0.12, with RMSPE between 2.94 and 4.68 kg DMI/d. Most prediction error was due to slope (29-87%) and mean (1-67%) biases. The results show considerable reranking between 3D_DMI and GreenFeed-derived traits, suggesting these technologies capture different aspects of cow metabolism and efficiency. The weak predictive relationship and limited concordance between 3D_DMI and equation-based DMI indicate poor agreement between the two approaches under commercial production conditions. Further work is needed to clarify how these technologies may complement each other for large-scale phenotyping and selection of feed-efficient dairy cattle.

SipControl: High-resolution pressure sensing to characterize granular sucking behavior in automated calf feeders

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Current data acquisition in automated calf feeders provides limited insights into the specific drinking behavior of calves. While parameters such as milk intake, visit frequency, and average drinking speed are recorded, the direct biomechanical interaction at the teat remains unmonitored. This granular sucking behavior enables advanced evaluations of individual animal health, ethology, and behavioral traits. SipControl utilizes high-frequency pressure sensors to capture individual sucking events, closing the gap between cumulative intake data and real-time behavioral monitoring. The objective of this technical approach is to demonstrate the seamless integration of SipControl into automated feeding systems and to visualize high-resolution sucking patterns at the teat. By transforming raw pressure signals into interpretable data streams, this method establishes a novel digital framework for future research in calf health, behavior, and precision livestock farming. The prototype of SipControl is integrated into a commercial calf-rearing facility in Lower Saxony. The farm manages an average of 300 calves. Since September 2025, the system has been continuously recording drinking visits in real-time for approximately 50 calves per batch. The animals, predominantly male Holstein-Friesian calves, enter the facility at four weeks of age. The nursing phase lasts 52 days. To date, the system has successfully captured over 5,000 individual feeding station visits per batch, providing a comprehensive dataset of sucking dynamics under practical farming conditions. The data acquisition is based on a high-frequency pressure sensor integrated directly into the milk line. The captured pressure fluctuations allow for the characterization of the calf's biomechanical sucking work, specifically the alternating phases of sub-atmospheric pressure generation and release, represented as a quasi-sinusoidal waveform. Data processing is handled on-site by a Raspberry Pi, which receives the raw sensor signals and transmits them into a time-series database. Simultaneously, log data from the automated feeder is captured in real-time and synchronized within the same database. Post-acquisition, the data is cleaned and pre-processed for subsequent feature extraction and behavioral analysis. Initial visualizations of the pressure data confirm the system's ability to capture fine-grained drinking dynamics. The sensor data allows the direct calculation of sucking frequency and the precise determination of individual sucking pauses based on inter-pulse intervals. By integrating data from the automated feeder, it is further possible to calculate the milk volume per swallow. Preliminary analysis suggests that amplitude height, when adjusted for teat material and wear, provides proxies for individual sucking strength. Furthermore, restless drinking behavior can be identified through metrics based on the mean, standard deviation, and coefficient of variation of the pressure signals. The data also reveals significant post-feeding activity at the teat after the milk allowance is exhausted, as well as activity during visits without milk entitlement. This technical approach demonstrates that pressure sensing is a viable and robust method for monitoring calf behavior. By making individual sucking acts quantifiable and synchronizing them with feeder logs, SipControl provides a novel data foundation that surpasses current industry standards. These findings offer significant potential for developing early warning systems for disease detection and addressing complex ethological questions regarding feeding management in automated systems.

Evaluation of non-invasive sensors for monitoring vital signs in dairy cows using pulse oximetry and infrared thermometry

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Continuous monitoring of physiological parameters in dairy cows is key to improving animal health, welfare, and farm productivity. However, traditional methods used to measure heart rate, respiratory rate, and body temperature require animal restraint and manual assessment, which can increase stress and limit their applicability for frequent monitoring under commercial farming conditions. Therefore, the development of non-invasive sensor-based technologies is gaining increasing interest within precision livestock farming systems. The aim of this study was to evaluate the feasibility of using low-cost, non-invasive sensors (i.e., a pulse oximeter and an infrared thermometer) to measure vital variables (i.e., heart and respiratory rates and temperature) in dairy cows under field conditions. The study was conducted on 40 multiparous Holstein dairy cows at different stages of lactation on a commercial dairy farm. Heart rate and respiratory rate were measured using a stethoscope and compared with measurements obtained using a pulse oximeter placed on both front and rear teats. Rectal temperature was recorded with a digital thermometer and compared with teat surface temperature measured using an infrared thermometer. The Pearson correlation coefficient was calculated using PROC CORR in SAS 9.4, and statistical significance was set at $P \leq 0.05$. The pulse oximeter measured heart rate at the teat level and showed a high correlation with stethoscope measurements, particularly in rear teats ($r = 0.79$ and $r = 0.85$, respectively; $P < 0.001$). However, the device was unable to obtain reliable measurements in pigmented teats, likely due to interference caused by melanin absorption affecting the photoplethysmographic signal. Respiratory rate measured with the pulse oximeter did not correlate with values obtained using the stethoscope ($P \geq 0.104$). Likewise, infrared teat temperature measurements were not correlated with rectal temperature ($P \geq 0.576$), suggesting that environmental factors and local physiological changes associated with milking may influence surface temperature readings. These findings suggest that pulse oximetry applied at the teat level may represent a promising approach for non-invasive heart rate monitoring in dairy cows within automated milking environments, although further technological adaptations are required to overcome limitations related to pigmentation and respiratory signal detection. Project carried out with a 2025 Leonardo Grant for Scientific Research and Cultural Creation from the BBVA Foundation.

Use of computed tomography to predict commercial lamb cut weights

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Accurate prediction of commercial cut weights underpins value-based trading in lamb supply chains. While hot standard carcass weight (HSCW) and GR tissue depth (110mm from the spine over the 12th rib) are widely used in Australia, their ability to discriminate variation in individual cut weights is limited. This study evaluated whether computed tomography (CT)-derived carcass composition traits improve prediction of commercial cut weights relative to GR tissue depth. 100 lambs from Meat & Livestock Australia's (MLA) Information Nucleus Flock, representing terminal, maternal, and Merino sire types, were slaughtered under commercial conditions. HSCW and GR tissue depth were recorded. Chilled carcasses were CT-scanned and dissected into commercial cuts following AUS-MEAT specifications. CT lean%, fat%, and bone% were quantified at whole-carcass and sectional levels. Log-transformed cut weights were modelled using linear mixed-effects models including HSCW and one of five fatness indicators (GR, carcass CT lean%, sectional CT lean%, carcass CT fat%, sectional CT fat%), with boner included as a random effect. Model performance was assessed via five-fold cross-validation using R^2 and RMSE as indicators of precision, and bias and slope deviation as indicators of accuracy. HSCW was the dominant predictor across all models. However, inclusion of CT traits, particularly sectional CT lean%, improved predictive precision for most cuts. For precision, the value of RMSE was as low as 0.03 to 0.05 (< 0.1% of cut weight for most cuts), however, the value of R^2 varied markedly among cuts (0.23–0.80), with larger, anatomically distinct cuts predicted most accurately. The CT lean component model provided the highest precision for the majority of fore, hind, and saddle cuts, and broadened predictive range of cut weights compared to GR model. Cross-validation confirmed stable performance in precision with minimal reduction in R^2 and minimal increase in RMSEP. For accuracy, although bias was low (bias < 3%), greater slope deviations were observed for smaller and anatomically complex cuts. These results demonstrate that CT-derived traits provide statistically robust and commercially meaningful improvements in predicting lamb cut weights, supporting more precise value-based marketing and carcass assessment systems.

What does outdoor access cost? A mechanistic analysis of energy expenditure in free-range slow-growing chickens

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Mechanistic nutrition models for broilers are mainly calibrated for fast-growing strains reared indoors under thermoneutral conditions, limiting their use for slow-growing genotypes reared in free-range systems. The aim of this study was to re-parameterize the daily time-step mechanistic growth model MsChick (Dukhta, 2023; DOI: 10.54598/002640) for slow-growing chickens with outdoor access. MsChick is a mechanistic "white-box" framework that predicts daily body weight (BW) based on feed characteristics and daily feed intake (DFI). It represents the daily partitioning of ingested energy and amino acids between maintenance, protein deposition and lipid deposition, thereby providing physiologically interpretable parameters. In the current study, we used individual daily performance data (BW and DFI) measured with high-throughput electronic feeders. These data were collected over two summer flocks (2022–2023) in males and females of the slow-growing S77N strain, reared either indoors or with outdoor access from day 28 (approximately 300 birds per sex × year × housing condition; Chaumont et al., 2025; DOI: 10.1093/jas/skaf157). Observed DFI values were used as model input to estimate, for each bird, three interpretable parameters controlling growth potential and basal maintenance cost: mean feather-free body protein deposition (meanBPD in g/d), protein maturity rate (precocity in d^{-1}), and fasting heat production (FHPinit in kJ/kg $BW_{0.70}$/d). The adjustment procedure minimised the difference between observed and predicted BW. In this first step, the additional cost of activity and thermoregulation (αAT) was fixed to the MsChick indoor default (αAT,IN = 33% of FHPinit). As expected, males exhibited lower precocity than females (0.031 vs 0.036 d^{-1}) but higher meanBPD (6.2 vs 4.1 g/d) and higher FHPinit (340 vs 294 kJ/kg $BW_{0.70}$/d), consistent with their higher BW and protein turnover. While indoor birds were reared under thermoneutral conditions, birds reared with outdoor access were exposed to more variable climatic conditions and could be more physically active. To capture this extra energy cost (i.e. activity + thermoregulation), we re-estimated an outdoor coefficient αAT using a two-step approach. First, total maintenance-related expenditure was derived from the energy balance (energy intake minus retained energy in proteins and lipids, and heat increment of feeding). Second, basal maintenance cost was computed by applying the indoor mean FHPinit value for each sex × year group to each bird according to its $BW_{0.70}$. The difference between total maintenance-related expenditure and basal maintenance cost was thus attributed to activity + thermoregulation and expressed as αAT,out as a percentage of FHPinit. With this approach, αAT,out was estimated to be higher than the indoor default value. It averaged about 39% and 45% of basal maintenance cost in males and females, respectively, exceeding the indoor default value. Moreover, in outdoor-access birds, combining total maintenance cost (calculated as FHPinit × [1 + αAT,out]) with abdominal fat percentage explained a large fraction of feed conversion ratio variability ($R^2 = 0.84$), whereas each predictor alone explained only a limited proportion of the variability ($R^2 < 0.40$). This work provides a first parameter set for slow-growing birds and a transferable calibration framework for representing outdoor-related energetic costs in mechanistic models while preserving interpretability and providing a basis for future scenario analyses (e.g. differing outdoor activity levels or environmental conditions).

Early prediction of growth dynamics and feed conversion ratio using random forest regression models in growing pigs

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In precision pig nutrition, high-frequency behavioural data generated by automated feeder systems create opportunities for growth-modelling as a dynamic, state-dependent process rather than as a static endpoint. The objective of this study was to develop and evaluate a trajectory-based autoregressive modelling framework capable of predicting week-to-week body weight transitions and estimating feed conversion ratio (FCR) from behaviour-derived proxy variables. The emphasis was on methodological robustness and transferability across independently collected datasets. Behavioural descriptors were calculated from raw automatic feeder records collected across multiple European datasets, including meal size, visit size, feeding frequency, total visits, total meals, meal duration, feeding rate, and the irregularity index of feed intake (IIFI; Salgado et al., 2021). Pigs visited the feeder on average 15–20 times per day, resulting in approximately 1,200–1,700 feeder events per animal during a 12-week fattening period. This generated a high-dimensional, longitudinal dataset characterised by repeated measures, behavioural heterogeneity, temporal dependency, and nonlinear interactions. To integrate behavioural organisation with physiological performance, individual body weight was recorded weekly. To model growth as a transition process, a trajectory dataset was constructed by pairing each observed body weight at week t (BW(t)) with the subsequent measurement at week t+1 (BW(t+1)). This formulation represented an explicit autoregressive structure in which the future state depends on current physiological status and behavioural descriptors. Subsequently, random forest regression (500 trees, bootstrap aggregation) was applied to predict BW(t+1) using BW(t), relative production week (time covariate), and selected behavioural predictors with low missingness. Predictors exceeding predefined missingness thresholds were excluded, remaining gaps were median-imputed, and models were fitted only when a sufficient number of week-to-week transitions was available. Model performance was quantified using R^2 and RMSE across consecutive weekly transitions. In parallel, FCR was calculated as total feed intake (kg) divided by body weight gain (kg). A separate random forest model was trained exclusively on behavioural descriptors to predict FCR with moderate but consistent accuracy across datasets. The results demonstrate that short-term growth progression can be robustly described using a structured ensemble-based autoregressive framework integrating physiological state and feeding behaviour organisation. This supports the hypothesis that intake structure and behavioural variability encode mechanistic information associated with inter-individual differences in feed efficiency. The stability of predictive performance across independent data origins further confirms the transferability of the modelling approach. The proposed framework provides a scalable analytical structure for transforming high-frequency behavioural data into biologically interpretable growth trajectories and feed efficiency indicators within precision livestock systems.

Opportunity for welfare breeding: An automated registration system to record foraging activity of individual pigs as an indicator trait for positive welfare

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Welfare breeding in pigs traditionally refers to selective breeding strategies to reduce pigs' negative experiences (e.g. lameness, tail biting, piglet morality). The WelBredPOrg project challenges this philosophy and argues that welfare breeding should also include selectively breeding for motivation to express natural behaviour related to positive experiences. Therefore, we aim to develop an automated system that can register natural foraging behavior of individual grower pigs. Data were recorded on an organic commercial finisher farm in Denmark. Each stable unit housed ~350 pigs and had both indoor and outdoor areas with straw bedding and sawdust beds, a concentrate feeder with an entrance gate with a scale, and a designated area in the outdoor area where roughage (i.e. grass silage) was provided ~5 times a day using an automated feeding robot. Individual pig body weight was recorded every time the pig entered the concentrate area. Antennas installed on the walls near the roughage area received signals from pigs' UHF-RFID ear tags. Signals were recorded up to ~2m from the wall. After each roughage provision, pigs were registered as (not) present for six consecutive 5min-intervals (present: ≥1 RFID-registration). Data from >3000 pigs were collected from October 2024 through December 2025. Preliminary results showed variation between pigs in how often they visited the roughage area after a roughage provision event (μ=35%, min=10%, max=80%). There was a positive correlation (r = 0.87 to 0.92) between the time a pig spent in the roughage area after roughage provision and how often pigs attended a roughage provision event. Throughout the entire growing period, pigs were most active in the late afternoon and evening (3:00 PM – 6:00 PM), and least in the morning (6:00 AM). No clear correlations were observed between average daily gain and a pig's activity in the roughage area (r = -0.20 to 0.30). Genetic parameters (h^2 and rg) will be estimated for activity in the roughage area and the average daily gain. This study presents a novel approach to use an indicator of natural behavior to selectively breed for enhanced positive welfare in pigs.

Impact of bronopol preservation on milk metabolomic stability

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With increased implementation of robotic milking systems, cows are milked at irregular intervals, which complicates the immediate freezing of milk samples. Since raw milk rapidly undergoes biochemical changes post-milking, there is uncertainty regarding the stability of metabolites when bronopol-preserved samples are collected at varying times and temporarily stored prior to freezing. Assessing bronopol's effectiveness under these circumstances is crucial for accurate milk profiling in contemporary dairy operations, where consistent metabolomic markers are vital for developing milk-based tools used for precision feeding and animal health evaluation. The objective of the project was to evaluate how bronopol preservation and short-term room-temperature exposure (0 h vs. 16 h) influence the metabolomic profile of lyophilized raw milk. Bulk milk (~50 mL) was collected over three days from a commercial dairy farm in Vaud, Switzerland, frozen at −20 °C, thawed, homogenized, and divided into four 10 mL aliquots per day. Half of the aliquots received bronopol following the manufacturer's recommendation. Within each treatment (with and without bronopol), half of the aliquots were immediately stored at −80 °C, while the remains were incubated at 25 °C for 16 h before −80 °C storage. Frozen samples were thawed at room temperature; for each sample, 5 aliquots of 1 mL were prepared and lyophilized, then reconstituted using 1 mL deionized water, vortexed and centrifuged at 15'000×g at 4°C for 20 min. Supernatant was then mixed with a buffer containing internal standards and analyzed by NMR for metabolites profiling. The experiment followed a randomized design, with treatments arranged in a factorial structure consisting of two factors: bronopol use (with or without) and incubation time (0 h or 16 h). Within the metabolomic profile, we identified 45 different metabolites. PLS-DA analysis showed strong discriminatory performance (R2Y = 0.93; Q2 = 0.788), explaining 56.9% of the X-variance, where bronopol treatment drove the primary separation along Component 1, largely influenced by bronopol concentration, while incubation at 25 °C contributed to additional variation along Component 2. Notably, when bronopol was added, samples incubated for 16 h did not differ statistically from their non-incubated counterparts, indicating substantial stabilization of the metabolomic profile. ANOVA supported these multivariate patterns, identifying a significant bronopol × incubation interaction for bronopol concentration. In addition, bronopol blocked the strong 16-h increases in fermentative metabolites observed in untreated milk, including lactic acid (~19-fold), pyruvate (~8-fold), acetate (~6-fold), and acetoin (~2-fold), indicating effective inhibition of fermentation. These findings demonstrate that bronopol stabilizes the milk metabolome during short ambient-temperature exposure, supporting its use when immediate freezing is not feasible. This contributes to more robust metabolomic monitoring pipelines for precision feeding and on-farm decision-support systems.

Pilot GWAS of male reproduction traits in Slovak farmed Rainbow trout

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Rainbow trout (Oncorhynchus mykiss, Walbaum 1792) represents one of the most important aquaculture species in Slovakia, with a long-standing tradition of intensive and semi-intensive farming, contributing substantially to national fish production and local food supply. As reproductive efficiency is a critical factor in aquaculture production systems, identifying genomic regions associated with male reproduction traits may support the optimisation of effective selective breeding strategies in this species. Thus, this study aimed to conduct a pilot genome-wide association study (GWAS) to identify genomic regions and certain genetic variants associated with male reproduction traits in a farmed rainbow trout population from Slovakia. Nuclear DNA from 20 males was genotyped using the GGP Rainbow Trout 20K array, and after standard quality control, the dataset comprised 17,329 informative SNP markers for each individual. The four reproduction traits, sperm concentration (10^6/ml), progressive motility (%), total motility (%) and immotile sperm (%), were measured directly on the farm as part of the routine annual evaluations of broodfish. The GWAS was performed for each trait separately using univariate linear mixed models, and the significance of associations was assessed via likelihood ratio tests. Candidate SNPs were identified using a false discovery rate (FDR) threshold of <0.1 and annotated by detecting the closest protein-coding genes (Ensembl BioMart, genome assembly USDA_OmykA_1.1). Sperm concentration in the studied broodfish averaged 23,200.35 $\times 10^6$/ml (SD = 5,066.07; range: 8,720–30,844), while progressive motility was on average 61.06 % (SD = 10.61%; range: 45.66–85.92%). Average total motility was 63.78 % (SD = 9.48%; range: 49.79–85.92%), and the proportion of immotile sperm was 36.22 % on average (SD = 9.48%; range: 14.08–50.21%). All four male reproductive traits showed relatively low heritability, with the majority of phenotypic variation explained by environmental (residual) factors. GWAS identified several significant SNPs associated with sperm concentration and sperm motility traits, with some loci affecting multiple motility traits simultaneously. Annotation of these SNPs revealed proximity to genes involved in neuronal regulation, cell signalling, and sperm development, including sv2bb, synm, SHANK3, ide, opcml, MCTP1, EDIL3, adora2aa, and sox6, while a few loci were unassigned. The detected SNPs represent potential candidate markers for selective breeding programs, enabling the identification of males with favourable alleles to enhance sperm concentration and motility, thereby improving fertilisation success and overall reproductive efficiency in farmed rainbow trout.
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Modelling post-farrowing body weight in sows from gestation and litter data

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Regular weighing of sows during the reproductive cycle is useful to assess the relevance of feeding management. When available in commercial farms, manual scales are used when sows are moved between gestation and lactations rooms, while automatic scales are installed in collective pens in the gestation unit. Thus, body weight at farrowing usually corresponds to data collected at the latest a few days before farrowing (stage G, days after the first insemination) and provides the total BW of the sow, including reproductive compartments. In this study, modelling was considered to estimate the sow's weight after farrowing (BWAF). Based on the literature that describes the growth of reproductive compartments, two nested models were built. The dynamic mechanistic approach allows the BWAF to be calculated from the total body weight at stage G by subtracting and adding the estimated reproductive compartments (i.e., uterus, placentas, fluids, foetuses, and mammary glands) at each stage to reflect their changes between stage G and farrowing. The first model (MOD1) estimated BWAF without accounting for maternal energy balance between stage G and farrowing, whereas the second model (MOD2) included body energy variation in maternal tissues, assuming an energy content of 8.37 MJ per kg of BW (the lowest value found in the literature for Large White x Landrace sows). The inputs were stage G, total body weight at stage G (BWG), gestation length, litter size and weight at birth, and, for MOD2, metabolizable energy (ME) supplied between stage G and farrowing calculated from the ME content of the feed and the daily ration. Models were evaluated using data collected in a demonstration farm from 1,067 sows weighed between 90 and 94 days of gestation and again within 24 hours after farrowing. Mean observed BWAF was 274 ± 40 kg. Predicted BWAF averaged 244 ± 37 kg with MOD1 and 270 ± 33 kg with MOD2. Including energy balance markedly reduced the prediction bias from 30 to 4 kg, the root mean square error of prediction from 33 to 15 kg, and the mean absolute error from 30 to 12 kg. With a 5% margin of error, the model MOD2 is accurate enough to estimate BW dynamics at herd level and support interpretation of routine BW measurements on commercial farms. However, its accuracy remains too low to allow for individual characterisation of sows in research contexts. Given genetic changes in prolificacy and body composition of sows, accuracy could be improved by updating knowledge on the growth of the reproductive compartments and on the maternal energy retention.

Analysis of fecal particle distribution in dairy cows with different assessment approaches

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Fluctuating feed intake, changes in the nutritional content of silage within a silo, and potential selection during feed intake can lead to deviations between the actual ration consumed and the calculated ration. However, the content of various carbohydrates such as starch or NDF must also be taken into account as an indicator of the ration. Against this background, the use of various key figures from feed control to monitor dairy cows is essential. Among the multitude of parameters, the evaluation of manure in dairy cows is a good option for individual animal-related or group-related analysis, as the very good mixing in the digestive tract and the longer retention time mean that a representative sample can be assumed. For the present study, based on the carbohydrate trial conducted by LLG Iden in 2022, manure samples from the dairy cows in the two feeding groups were analyzed on various dates to determine potential differences in the animals' digestion. The focus was on whether differences in fecal particle distribution could also be observed between a starch-rich and an NDF-rich TMR. The second part of the study was to examine possible differences between the fecal particle distribution of cows in the high-yield phase, cows at the end of lacation and dry cows. For the present investigations, the "RETSCH AS 200 BASIC" system was used in the LLG Iden laboratory. The following sieves were used: [1] 4 mm, [2] 1 mm, and [3] 0.125 mm. The fecal samples were poured onto the top sieve and doused with approximately one liter of water. On average, 28% of the fecal particles were larger than 4 mm, but there was a significant difference between the two feeding groups, with higher proportions in the fiber group. In contrast, the particles on the 1 mm sieve were significantly higher in the starch group (21%) than in the fiber group (16%). However, no statistically significant differences between the two feeding groups could be detected on the smallest sieve. A comparison of the lactation stages showed that the cows in the high-performance group had the highest 4 mm sieve percentage at 37%. In contrast, dry cows had the significantly highest proportion on the 0.125 mm sieve at 64%, compared to 53% for the other two groups. In summary, it can be concluded that sieving manure is very useful for detecting differences in feeding trials and also between different ration types. For further investigations and to increase the informative value of the fecal screenings, it should be examined whether the proportion of washed-out feces in the weighed quantities should also be taken into account.

Methane Yield Integrated into Feeding Strategies as a Measure of Production Efficiency in Tropical Commercial Dairies: Case Study in Tamil Nadu, India

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A feeding trial was conducted on a commercial farm in Tamil Nadu, India, to investigate the use of enteric methane emissions (CH4) as a metric of production efficiency in tropical dairy systems. The study was carried out from October to December 2024, during the heavy-rain monsoon season. Twenty lactating cows of the indigenous Kankrej cattle, in their third lactation and aged 6 -7 years were selected from a herd of 200 animals using a randomized block design (RBD) based on milk yield (Group 1: <10L/day; Group 2: >10L/day). The trial was set up as a repeated-measures design with two dietary phases, where milk yield and CH4 emissions were recorded over five consecutive days in each phase. The diet consisted of a maize silage-based TMR with a concentrate, crude protein (CP) levels of 11.96% in the first phase and 14.62% in the second phase. A 14-day adaptation period was included between the two dietary phases prior to the second phase of measurements. Enteric CH4 emissions were measured directly using a laser methane detector (LMD). Methane emission intensity, milk yield and methane production were analyzed using a linear mixed-effects model with diet (original vs. adapted), lactation stage and milk yield group as fixed effects. Raw means (SD) of CH4 production and milk yield were 150.6 (76.4) g/day and 8.9 (3.37) L/day respectively. Diet had a significant effect on milk production and CH4 ($p<0.05$). Following the adapted diet, both CH4 production and milk yield increased significantly ($p<0.05$) compared with the original diet (LS means, 175.4 ± 9.26 g/day vs. 114.4 ± 9.33 g/day for CH4 ; 10.2 ±0.61 vs. 8.5 ±0.61 L/day for milk yield). Alternatively, CH4 emission intensities remained similar (LS means, 0.02 ± 0.003 vs. 0.017 ± 0.003 kg CH4/kg milk; $p>0.05$) in the original and adapted dietary phases respectively. Lactation stage did not significantly influence CH4 emissions or milk yield. The results show that a higher CP-diet increased both CH4 production and milk yield without significantly increasing CH4 emission intensity. These findings suggest that methane emission intensities could serve as a useful metric when designing feeding strategies aimed at balancing emission reduction and dairy productivity. This is especially important in countries such as India, where high milk demand coincides with a limited feed resource. In conclusion, the study highlights that integrating methane-based efficiency metrics into feeding practices can support sustainability in dairy farms by maintaining methane emissions intensities while improving milk production.

"Collar-Tag": A Novel, Rapid, and Secure Electronic Identification Method for Lambs

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Accurate identification of newborn lambs represents a critical step in sheep production systems for reproductive management, performance recording, traceability, and genetic evaluation. Despite the widespread adoption of official electronic identification systems in Europe, early-life recording on farms often still relies on paper-based notes or delayed digital entry, increasing the risk of mismatches between lambs and dams, transcription errors, and data loss during the lambing period. This issue is particularly relevant in prolific flocks and in large-scale operations, where rapid and reliable identification at birth is essential. This work presents a rapid, low-cost electronic method for temporary identification and early-life data collection in newborn lambs, using a microchip-equipped collar (Collar-Tag) combined with programmable electronic readers. The system is designed to bridge the time gap between birth and the insertion of the official national bolus (BDN), replacing traditional paper-based recording and allowing immediate digital registration of key maternal and neonatal information from birth until official identification. Each Collar-Tag links a visible progressive number to an ISO electronic ID, enabling fast field recognition and error-free association through a handheld reader. Data are collected using customized templates created with herd-management software (Rumisoft, Tru Test), transferred to the reader, and downloaded in TXT or CSV formats for further processing and integration into farm databases. Regular updates of equivalence files ensure accurate linkage between temporary collar IDs and their dams throughout the lambing period. Additional functionalities include automated weight recording via a scale equipped with an integrated reader, supporting continuous growth monitoring and management decisions such as selection of replacement or slaughter destination. Within the framework of precision livestock farming and digital traceability in small ruminants, this approach improves data accuracy, reduces labor time, and provides a standardized, fully digital workflow suitable for large and small sheep farms aiming to enhance reproductive and performance monitoring. The system is developed for sheep production and is relevant to animal science disciplines including herd management, breeding, and livestock informatics. Stakeholders potentially benefiting from this tool include farmers, breeders' associations, genetic evaluation bodies, advisory services, and institutions involved in livestock traceability and digital innovation. Presenting this contribution at EAAP 2026 aims to stimulate discussion on practical and scalable solutions to improve early-life data quality and management efficiency in European sheep production systems.

Can feed color drive feeding behavior in piglets?

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Feeding behavior in weaned piglets is influenced by multiple factors, but the contribution of feed appearance, and specifically feed coloration, to feed preference has received limited attention. The aim of the study was to evaluate the effect of feed color (red, green, and blue) on feed preference in weaned piglets. A total of 108 weaned piglets ([Large White × Landrace] × Pietrain) were allocated to 36 pens (3 piglets per pen) in two rooms (24 and 12 pens, respectively). A double-choice preference trial was conducted following a randomized complete block design with 9 blocks and four feed comparisons per block. Piglets were offered the choice between a control (uncolored) diet versus blue-, red-, or green-colored feeds obtained by using the feed colorants Patent Blue V 85%, Red 40 CONACERT, and Mint Green 839 CONACERT, respectively (all provided by PROQUIMAC PFC, Barcelona, Spain). Feeds were offered in pelleted form. Disappearance, over 3 days, of the two feeds offered to each pen was measured at pen level and their relative preference was expressed as percentage of total feed intake. Feed preferences were analyzed with the GLM procedure of SAS. Piglets showed significantly higher preferences for the green- and red-colored feeds than for the control diet (74 vs. 26% and 76 vs. 24%, respectively; $P < 0.05$). No difference in preference was observed for the blue-colored feed (54 vs. 46%; $P > 0.05$). To assess whether these effects of the colorants were certainly due to changes in color, and not to other possible sensorial properties of the colorant (i.e. taste or odor), the preference test was performed again under the same conditions, with the exception that it was performed under complete darkness. In addition, to minimize carryover effects, piglets were not re-exposed to the same feed color across testing periods. Under dark conditions, no significant differences in preference were observed between the green- or blue- feeds and the control diet (53 vs. 47% and 53 vs. 47%, respectively; $P > 0.05$). On the other hand, piglets maintained a strong preference for the red-colored feed in absence of light (72 vs. 28%; $P < 0.05$). The persistence of red feed preference under dark conditions suggests that in the case of Red 40 CONACERT dye, sensorial properties other than color may have been responsible for the preference observed. It is concluded that the green color of feed improves feed preference in weaned piglets.

Chronic stress reshapes the pig gut microbiome: insights from metaproteomics

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Chronic stress compromises pig welfare and productivity, mainly through the impact on the microbiota-gut-brain axis. While multi-omics is a promising tool for uncovering its underlying mechanisms, the application of metaproteomics to chronic social stress in pigs remains unexplored. The objectives of this study were to characterise how stress reshapes the functional activity of the pig gut microbiome and to identify metaproteomic signatures that classify the groups. At the end of the growing period, hair and faecal samples were collected from 60 two-month-old Duroc pigs that had been exposed to either chronic social stress (n=30; reduced space allowance and repeated mixing) or control conditions (n=30). Significant differences ($p<0.05$) in hair cortisol levels were observed between groups. Faecal samples were analysed using a genome-resolved metaproteomic approach. Metaproteomic profiles were generated using nano LC-MS/MS and searched against a custom database of 911,040 microbial genes derived from 484 metagenome-assembled genomes (MAGs) generated from the samples. This enabled the direct linkage of expressed proteins to their microorganisms and the quantification of active MAGs based on protein abundance. Discriminant and differential abundance analyses identified stress-responsive taxa and functions, with group discrimination accuracy based on individual proteins up to 99.51%. Functional profiling, through enrichment analysis and metabolic pathway reconstruction, revealed a clear distinction between the metabolic strategies of condition-associated MAGs. Discriminant MAGs that distinguished the stress condition included Treponema_D, Catenibacterium, and members of the Erysipelotrichaceae family. In contrast, those associated with the control condition were predominantly represented by PeH17, Roseburia, Butyricicoccus, and a novel species belonging to the Christensenellales order and the Lachnospiraceae family. The functional profile of the control-associated community was characterised by capabilities for short-chain fatty acid production, vitamin synthesis, and stress resistance, as well as significant metabolic dependencies, supporting a role for specialisation and symbiosis in a homeostatic, cooperative gut environment. In contrast, the stress-associated microbial ecosystem was defined by resource competition, including motility, diverse transport systems, and a more autonomous metabolism geared towards nutrient scavenging and utilisation under fluctuating conditions, aligning with a dysbiotic state. These findings demonstrate that chronic social stress drives a functional reorganisation of the pig gut microbiome, shifting it from a specialised, symbiotic consortium towards a competitive, dysbiotic community. The identified metaproteomic signatures provide highly accurate functional biomarkers for chronic stress, establishing a powerful tool for welfare assessment in pigs and offering translational insights into human stress-related dysbiosis.

Step into the Udder Zone – Identifying behavioural proxies of fallback piglets under heavy occlusion

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In pig production, larger litters have increased the risk of piglets dying from starvation arising from intensified teat competition, particularly in the first week of life. Clearly, such animals will appear as malnourished or 'fall-back' piglets prior to death. Early identification of fallbacks could enable targeted management interventions, yet routine weighing or behavioural observations are labour intensive and disruptive. Video recording offers a scalable and non-invasive alternative. Recent advances in Computer Vision (CV) have further increased the feasibility of automated behavioural monitoring in livestock systems. However, farrowing pens present suboptimal environments for CV because piglets are frequently occluded by housing structures and littermates, complicating continuous individual tracking and behaviour classification. We therefore explored whether behavioural differences during nursing bouts are associated with fallback risk using an observation approach designed to remain informative under heavy occlusion. Rather than relying on continuous tracking, the method exploits the distinct functional areas of the farrowing pen, based on the principle that piglets cannot teleport between zones. Specifically, we hypothesised that behaviour during nursing bouts, when scored manually using a simple zone-based ethogram, would be associated with fallback risk. Average piglet daily weight gain (ADG) during lactation was recorded by weighing the piglets of individually penned, permanently crated sows on days 1, 2, 6, and 14 postpartum. Piglet behaviour during the first eight nursing bouts after each weighing was scored from video recordings using an ethogram including: "in Udder Zone", "walking, "lying", or "out of sight". The Udder Zone was defined as a rectangular area surrounding the sow's udder from anterior to posterior shoulder and one piglet body length in width. Derived metrics summarised nursing bout participation, initiation, and teat fidelity. Piglets that died or fell within the lowest 15% of ADG within each litter × weighing interval were classified as fallbacks. Principal component analysis (PCA) was used to reduce correlated behavioural metrics into orthogonal components, which were subsequently analysed using generalised linear mixed-effects models. Preliminary analyses on three litters showed that PCA reduced the behavioural metrics to a dominant engagement axis (PC1), characterised by lower bout initiation and participation together with longer latencies and more re-entries into the Udder Zone. This engagement axis was associated with fallback risk ($P = 0.019$), with higher PC1 scores (reflecting reduced and less stable nursing engagement) corresponding to increased odds of growth failure. Additional behavioural components did not improve model fit and were not independently predictive. Within-litter standardised birth weight was not associated with fallback risk. Random intercept variance at the piglet level was large, indicating substantial intrinsic heterogeneity. These results suggest that simple, zone-based behavioural scoring captures a dominant nursing engagement dimension associated with fallback risk without requiring continuous individual tracking. Although we found substantial piglet-level heterogeneity in growth trajectories, such behavioural indicators may provide a biological foundation for future CV monitoring systems operating under the heavy occlusion conditions typical of commercial farrowing pens.

Influence of different sow and farrowing parameters on colostrum quality in a free farrowing system

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Colostrum is vital for neonatal piglets, with both quantity and quality determining survival and performance. Inadequate colostrum quality is a major risk factor for impaired health, growth and welfare. This study aimed to investigate the influence of sow and farrowing parameters on colostrum immunoglobulin (IgG) concentration. In total, 151 crossbred sows housed in free-farrowing pens were included. Sow-related data (parity, body weight, body condition score, backfat, birth induction, gestation length) and farrowing parameters (total piglets born, farrowing duration, obstetrical intervention, placental expulsion, litter and placental weight) were recorded. Ambient temperature and humidity were recorded within 24 hours prior to farrowing. Colostrum samples were collected after the expulsion of the first piglet and analysed for IgG concentration using a porcine-specific ELISA (Pig IgG ELISA, E101-104, Bethyl Laboratories Inc., Montgomery, TX, USA; dilution 1:1,000,000; intra- and inter-assay CV 5.4% and 4.4%), with parallel measurement by digital Brix refractometry. Linear regression with log-transformed IgG values was applied. Predictor variables were selected based on biological plausibility and descriptive analyses; the final model included total piglets born, parity, induction of farrowing, relative humidity, and the parity × induction interaction. Mean parity was 4.23 ± 2.46 (range 1–9), and mean litter size was 15.98 ± 3.55 piglets. Mean Brix value was 27.20 ± 2.95%, and mean colostrum IgG concentration was 66.57 ± 24.70 mg/mL. Brix values strongly correlated with ELISA IgG ($r = 0.66$, $p < 0.001$). The final model ($R^2 = 0.43$, $p < 0.001$) revealed significant effects of parity ($p < 0.001$), birth induction ($p = 0.001$), humidity ($p = 0.004$), and the interaction between parity and induction ($p = 0.034$). Higher parity was associated with increased IgG levels, whereas birth induction reduced IgG, particularly in gilts, with diminishing effects in older sows. A positive association between ambient humidity and IgG was observed, though its biological relevance is unclear. In conclusion, parity, birth induction, and environmental factors significantly influenced colostrum IgG. The negative impact of birth induction highlights the importance of farrowing management. On-farm colostrum assessment by Brix refractometry appears feasible for integration into sow and piglet management in free-farrowing systems.

Effect of early and late postnatal administration of two artificial colostrum formulas on piglets' growth performance, blood metabolic profile and intestinal health

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Artificial colostrum supplementation, provided after birth and during the pre-weaning phase, may enhance piglet resilience especially in large litters and in low-birth-weight piglets; however, its efficacy is inconsistent, partly due to compositional differences from sow colostrum. Notably, bovine-based formulas lack key bioactive compounds such as taurine and vitamin E, which are linked to improved metabolic function, intestinal health, and survivability. This study evaluated the effects of a standard bovine-based formulation (COL) or the same formulation enriched with taurine and vitamin E (PLUS), administered early (first 3 days after birth: d0-d2) and late before weaning (d26-d28), on piglet survival, growth, welfare, blood metabolic profile, and intestinal health. Fifty sows and their litters were allocated to 5 groups (10 litters/group): CO (no artificial colostrum); COL3 (COL d0-d2); COL28 (COL d0-d2 and d26-d28); PLUS3 (PLUS d0-d2) and PLUS28 (PLUS d0-d2 and d26-d28). Doses were administered by gavage d0-d2 and by feeders pre-weaning. At birth (d0), piglets were weighed and classified in body weight category (BWC): low (LBW, <1300 g), normal (NBW, 1300–1700 g), and high (HBW, >1700 g). Piglets' growth performance, ear lesions, and litter size were recorded until weaning (d28). At d28, 80 piglets (balanced for BWC) were euthanised for blood (Igs and untargeted metabolomics analysis), and jejunal sampling (morphometry, scoring of Cldn4, Ocln, and ZO-1 immunoreactivity (-IR), transcriptomics). Data were analysed using generalised linear and mixed models followed by ANOVA on R software. Metabolomic data were analysed using PLS-DA approach and RNA-seq differential analysis was performed with DESeq2, followed by KEGG-based Gene Set Enrichment Analysis. Sow performance and colostrum composition did not differ among groups. Neither COL nor PLUS improved overall growth and survivability compared with CO. However, COL28 piglets had greater body weight (d28) and higher ADG (d0-d28) than COL3 ($P < 0.05$), indicating a beneficial effect of late supplementation. All supplemented groups had reduced ear lesion scores at d5 and d28 ($P < 0.05$). Blood Igs were unaffected by treatment. Metabolomic analysis (1,066 metabolites) revealed treatment-related clustering (sPLS-DA), with COL groups diverging from CO more than PLUS groups. Jejunal morphometry was unchanged, but COL3 piglets showed lower Cldn4 and Ocln-IR ($P < 0.05$), suggesting impaired barrier integrity. Transcriptomic analysis identified 30 differentially expressed genes between COL3-LBW and COL28-LBW piglets (Padj < 0.05; log2FC >1.5), with suppressed basal transcription/metabolic pathways and activation of cytoskeleton-related pathways in COL3-LBW, consistent with epithelial stress. In conclusion, artificial colostrum supplementation did not improve piglets' growth and survivability; however, it improved piglet welfare by reducing ear lesions. Late administration of COL before weaning showed positive effects by enhancing growth and improving intestinal integrity compared with the early supplementation. Finally, taurine and vitamin E enrichment did not improve growth compared to COL.

Modulation of Dietary Acid-Binding Capacity by Limestone Flour Omission and Fumaric Acid Supplementation in the First 14 Days Post-Weaning: Effects on Pig Performance and Health

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The pig's ability to produce gastric hydrochloric acid in the early post-weaning (PW) period is limited. Therefore, reducing dietary acid-binding capacity to a pH of 4.0 (ABC-4) has been proposed to help maintain gastric acidity and improve growth and health PW. Limestone flour is the main calcium source in the diet and has a very high ABC-4 of ~12,932 meq/kg. Contrary to this, fumaric acid has a very low ABC-4 of ~-10,862 meq/kg. Complete PW diets can be formulated to have a low ABC-4 by omission and inclusion of high and low ABC-4 dietary ingredients, respectively. The objective here was to determine the effect of reducing dietary ABC-4 for 14 days PW on pig growth and health. At weaning (27 ± 1.02 days), pigs were formed into same-sex (entire male or female) pen groups of 10 pigs of even weight (8.2 ± 1.10 kg). Pen groups were blocked on sex and weaning weight. Within block, pen groups were randomly assigned to one of four dietary treatments (N=14 pen replicates per treatment) as follows: 1. With limestone flour (10g/kg; ABC-4 = 435 meq/kg; CONTROL), 2. Without limestone flour (0g/kg; ABC-4 = 305 meq/kg; LF-), 3. With limestone flour plus fumaric acid (21.5 g/kg to target a dietary ABC-4 of 200 meq/kg; FA+) and 4. Without limestone flour plus fumaric acid (10g/kg to target a dietary ABC-4 of 200 meq/kg; LF-/FA+). Experimental diets were fed from weaning until day (D) 14 PW after which common weaner (D14-43 PW) and finisher (D43-120 PW) diets were fed. Average daily gain (ADG), average daily feed intake (ADFI) and feed conversion ratio (FCR) were calculated, and statistical analysis was conducted using PROC MIXED in SAS. Treatment was included as a fixed effect with block included as a random effect and weaning weight, when significant in the model, was included as a co-variate. Titrations of diets revealed that actual ABC-4 values were 404, 281, 163 and 171 meq/kg for CONTROL, LF-, FA+ and LF-/FA+, respectively. There was a treatment effect observed for pig body weight (BW) at D14 PW ($P<0.001$) and ADG from D0-14 PW ($P<0.001$). Feeding FA+ resulted in a 5.3% increase in BW at D14 PW ($P<0.05$) and a 25.6% increase in ADG compared to control during the period from D0-14 PW ($P<0.05$). Feeding LF- resulted in a 7% increase in BW at D14 PW ($P<0.05$) and a 31.3% increase in ADG compared to control during the period from D0-14 PW ($P<0.05$). Feeding LF-/FA+ did not affect BW at D14 PW or ADG from D0-14 PW ($P>0.05$). There was a treatment effect observed for FCR from D0-14 PW ($P<0.001$). The LF-, FA+ and LF-/FA+ diets all improved FCR compared to CONTROL ($P<0.05$). There was no treatment effect observed for any growth parameter of interest at any timepoint from D14-120 PW ($P>0.05$). In conclusion, reducing calculated dietary ABC-4 by ~130 meq/kg by removing limestone flour from the formulation or by targeting a calculated dietary ABC-4 of ~200 meq/kg by including 21.5 g/kg fumaric acid in the diet both increased BW and ADG and improved feed efficiency during the first 14 days PW. However, reducing dietary ABC-4 to a target level of ~200 meq/kg by removal of limestone flour and fumaric acid addition to the formulation did not increase pig BW or ADG but did improve feed efficiency compared to the CONTROL during the first 14 days after weaning.

Effect of dietary inclusion of a by-product of Gelidium sesquipedale on post-weaning growth and health of pigs

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Gelidium is a red macroalga rich in bioactive compounds (polyphenols, sulphated galactans, dietary fibre) known to promote intestinal health and support immune status. A by-product was obtained after agar extraction from Gelidium sesquipedale via enzymatic hydrolysis and water extraction processes. The protein (27%), carbohydrate (31%), and sulphated polysaccharide content of the by-product may make it suitable for use as an alternative feed ingredient in pig diets. This study evaluated the effect of dietary inclusion of this Gelidium sesquipedale by-product in post-weaning (PW) diets on piglet growth, whole blood haematology and biomarkers of small intestinal health. At weaning (29±0.95 days; 9.3±0.05 kg), 126 pigs were formed into 30 pen groups containing 3 pigs and 18 pen groups containing 2 pigs. Pen groups were blocked on pen group size at weaning, sex (entire male and female) and weaning weight. Within block, pen groups were randomly assigned to one of three dietary treatments (n=16 pens/treatment): 1. Control diet; 2. Control + 1% Gelidium by-product (GEL1); 3. Control + 3% Gelidium by-product (GEL3). Pigs were fed the experimental diets from weaning until day (d)21 PW, after which a common weaner diet was provided until d44 PW. Individual pig weights were recorded at weaning (d0), d6, 14, 21, 27 and 44 PW. Feed disappearance was recorded for the intervals between pig weighings. At d6 PW, one pig from each pen containing 3 pigs (n=10 pigs/treatment) was euthanised to collect whole blood for haematology and intestinal tissue (duodenum, jejunum, and ileum) for histology and mRNA analysis. Pig growth was analysed using PROC MIXED with day as the repeated measure; haematology and mRNA expression were analysed using PROC GLIMMIX in SAS. Dietary treatment was included as fixed effect, block as random effect and weaning weight as covariate when significant in the model. Pen was the experimental unit for growth parameters, while individual pig was the experimental unit for haematological, histological and mRNA data. Dietary treatment did not affect body weight or average daily feed intake up to d44 PW. Feed conversion efficiency was improved for GEL1 compared to Control and GEL3 during the period d0-6 PW (P=0.05), with a similar tendency observed compared to Control during the period d0-44 PW (P=0.08).). Duodenal villus height to crypt depth ratio tended to be higher in GEL1 and GEL3 compared to Control (P=0.08), while ileal villus height tended to be higher in GEL1 compared to Control (P=0.09). Duodenal CLAUD4 was increased in GEL3 pigs compared to GEL1 and Control (P<0.01) with a tendency for it to increase in GEL3 compared to Control in the jejunum (P=0.10). This may be due to a local epithelial response to higher levels of Gelidium derived bioactive compounds. In summary, dietary inclusion of the Gelidium by-product at 1% or 3% in post-weaning diets had minimal effects on pig growth, blood haematology or intestinal function. Therefore, this by-product can be effectively used as an alternative feed ingredient in post-weaning pig diets at inclusion levels of up to 3%.

Effects of the combination of potentiated zinc oxide and monovalent copper in low acid-binding capacity-4 diets on growth performance and plasma zinc and copper concentrations

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This study evaluated the effects of the combination of potentiated zinc oxide (pZnO, HiZox, Animine Precision Minerals, Annecy, France; source with small aggregates and agglomerate particles) and monovalent copper oxide (Cu2O, CoRouge, Animine Precision Minerals) as alternatives to pharmacological levels of ZnO in low acid-binding capacity (ABC)-4 diets on growth performance and plasma Zn and Cu concentrations of nursery pigs. A total of 360 nursery pigs (18 ± 1 d old; initially 5.7 ± 0.2 kg) were allotted within three body weight (BW) blocks to one of six treatments (12 pens/treatment; 5 pigs/pen). Treatment diets were fed in phase 1 (d 0 to 10), phase 2 (d 10 to 24), and phase 3 (d 24 to 46) diets. Treatments were arranged in a 3 × 2 factorial with main effects of Zn: low (150 mg/kg) and high (800 mg/kg) levels of Zn from pZnO for both phase 1 and 2, and pharmacological levels of Zn from standard ZnO (Maximo720; Zinc Nacional, Monterrey, Mexico; source with large and dense particles) at 3,000 and 2,000 mg/kg for phases 1 and 2, respectively, and Cu levels:18 vs. 150 mg/kg of Cu from Cu2O. In phase 3, treatment diets included two different Cu from Cu2O levels (18 and 150 meq/kg) with 150 mg/kg of Zn from pZnO. The ABC-4 levels of basal diets were 199 and 248 meq/kg for phases 1 and 2, and 437 meq/kg for phase 3. Neither Zn × Cu interactions nor Zn and Cu main effects were observed in d 10 and 24 BW. However, Cu main effects were significant ($P \leq 0.05$) for d 46 BW and phase 3 average daily gain (ADG), in which pigs fed high-Cu2O diets had greater BW and ADG, compared to those fed low-Cu2O diets. For d 46 plasma Zn and Cu concentrations, neither Zn × Cu interactions nor Zn and Cu main effects were observed. In conclusion, without sanitary challenges, lower dietary inclusion of pZnO had comparable effects on piglets' growth to pharmacological levels of ZnO in low ABC-4 diets, while the inclusion of a high level of Cu2O improved growth performance without influencing the plasma Cu concentrations during phase 3.

Effect of Lactiplantibacillus Plantarum on nitrogen, calcium and phosphorus balance in weaned piglets challenged with Fumonisins B1

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Abstract Fumonisin B1 (FB1), a mycotoxin produced by Fusarium fungi frequently contaminating maize and maize-based feeds posing health risks to swine by disruption of intestinal integrity and nutrients metabolism. Although dietary probiotic Lactiplantibacillus plantarum (L. plantarum) modulates nutrients utilization, its efficacy in FB1-intoxicated conditions remain undocumented. The aim of the study was to investigate the effect of L. plantarum on nitrogen (N), calcium (Ca) and phosphorus (P) balance in weaned piglets challenged with FB1. Twenty-four piglets aged 28 days with an initial body weight of 7.92 ± 0.15 kg were randomly assigned to four dietary treatments groups: commercial feed (Control, C), 50 mg/kg FB1-contaminated commercial feed (F), commercial feed supplemented with 1×10^9 CFU/pig/day L. plantarum (LP), 50 mg/kg FB1-contaminated commercial feed supplemented with 1×10^9 CFU/pig/day L. plantarum (FLP). The experimental period included 14 days of acclimatization and 28 days of trial. Statistical analysis was by GLM procedure of SPSS software version 29.0.1.0. Results showed no significant interaction effect ($p>0.05$) between FB1 and L. plantarum. Similarly, N intake, apparent total tract digestibility (ATTD), absolute and relative N retention was not affected by dietary treatments ($p>0.05$). However, a significant main effect of FB1 was reported in Ca and P metabolism ($p<0.05$). Pigs challenged with FB1 showed a significantly ($p=0.022$) lower ATTD of Ca ultimately decreasing its retention by 5.3 % points of intake ($p=0.038$). While P ATTD remained unaffected ($p>0.05$), pigs challenged with FB1 showed a significantly higher urinary P excretion ($p<0.001$) which in turn led to a significant reduction in the rate of retained P to intake ($p=0.043$) and digested P ($p<0.001$). Supplementation of probiotic L. plantarum did not mitigate the negative effects of FB1. The results confirm that FB1 impact on P balance is post-absorptive. The significant reduction in the Ca ATTD and high P urinary excretion in FB1 challenged piglets in our study, suggests disruption of Ca:P homeostasis which may dispose the pigs to skeletal demineralization with time warranting future investigation. Keywords: weaning pigs; Fumonisin; digestibility; retention; Lactiplantibacillus plantarum
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Evaluating social genetic models in pigs with precision feeding data and stochastic simulation

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Including social genetic effects into breeding strategies can improve accuracy of genetic evaluations, limiting the negative-competitive effects in group-housed pigs. Technologies applied in pig production systems allow a characterization of social interactions in groups of pigs. This study aimed to assess (1) the impact of including social effects versus a classical animal model in the genetic evaluation of productive traits in a limited real population and simulated data, and (2) to evaluate differences in performance of a classical binary social model and a model including the level of interaction between animals. Real data included 1,195 Iberian pigs housed in pens of 12 animals, with feed intake recorded through automatic feeders. Pigs were housed in pens with an average of 13 pigs per pen. Animals entered the testing period with an average initial weight of 31.4 kg and finished with 80.4 kg. In parallel, a stochastic simulation was conducted to generate 10,080 phenotypes with direct and social genetic effects. Animals were allocated into 840 pens of 12 individuals, with an average within-pen relatedness of 0.068. Direct and social breeding values for founders were sampled, assuming a direct heritability of 0.2 and a direct–social genetic correlation of −0.3. Breeding values of descendants were generated as the mid-parent value plus a Mendelian sampling term. The effect of contemporary mates on the phenotype of animal i was obtained as $\sum ws$, where w is the intensity of interaction between i and each of its (n-1) mates, and s is the social breeding value of each contemporary mate. The interaction was simulated using an exponential weighting function of standardized social breeding values: where $\beta=3$ controls the degree of heterogeneity. This formulation results in lower displacements by animals with higher social breeding values, and vice versa. Ten independent replicates were simulated, and variance components were computed for each replicate. Three models were compared for daily feed intake of both real and simulated data: an additive genetic model (GM), a classical binary social model (SBM), and a social model with continuous interaction intensities (SCIM). While SBM assumed equal interaction among contemporaneous animals, SCIM incorporated continuous interaction intensities, quantified (in real data) as the frequency of displacements between pairs of animals. Displacements were defined as feeder visits in which the interval between consecutive animals was less than 60 seconds together with a reduction in feed intake of the displaced animal. Variance component estimation was performed with blupf90+. Correlations between estimated and true breeding values were assessed for each replicate and averaged across replicates to compare prediction ability between models. Results from GM model showed that social genetic variance was largely absorbed into the pen effect, being in line both real and simulated data. While EBV predictions were similar across models (0.77), SCIM improved the estimation of social values (from 0.59 to 0.63) and total genetic values (from 0.52 to 0.57), reflecting the benefit of including interaction intensities. These findings suggest that modeling heterogeneous interaction intensities produces more realistic results and helps mitigate the classical confounding between pen and social effects. Automatic feeder data offers a scalable way to quantify intensities without additional labour or cost. Nonetheless, further studies using larger real populations are needed to validate the application of social genetic models with intensity. Funding: COST Action CA22112 (EU-LI-PHE).

Enrichment with live insect larvae (Hermetia illucens) on productive results in slow- and fast-growing broiler chickens

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Fast-growing broiler genotypes are raising many concerns about animal welfare and meat quality defects. These include a high incidence of growth-related myopathies such as white striping, wooden breast, and spaghetti meat. Together with reduced locomotor activity and behavioural expression, these issues have raised ethical concerns. Consumer awareness is growing, which has led to initiatives like the European Chicken Commitment, encouraging the use of slower- and medium-growing genotypes in alternative production systems which can also use insect, such as live Hermetia illucens (HI) larvae, as promising feed ingredient with high nutritional value and potential environmental benefits. Providing live HI larvae can also stimulate natural foraging behaviour, which could have a positive effect on productive performance and animal welfare. Consistently, to evaluate the effect of live HI larvae supplementation on the performance and meat quality in fast-growing and slow-growing broiler chickens, a total of 498 one-day-old male chicks (325 Ross 308 and 173 Ranger Savanna, Aviagen, Huntsville, AL) were housed in 12 collective pens (Ross, 54 birds/pen and Ranger, 29 birds/pen, 3 pens per experimental group) fed a control diet and receiving or not 10% HI live larvae (on the litter) until commercial slaughtering (42 and 59 days of age for fast-growing and slow-growing chickens, respectively). Data were analysed using a mixed model with live-larvae enrichment and genotype as a fixed effects and pen as a random effect. Live weight (3405 g vs. 4543 g; $P<0.001$) and cold carcass weight (2479 g vs. 3347 g; $P<0.001$) were higher in slow- compared to fast-growing chickens. The rates of breast yield (40.3% vs 39.4%; $P<0.05$) and pectoralis major (26.2% vs. 25.7%; $P=0.06$) and the occurrence of wooden breast (35.4% vs 15.6%; $P<0.001$) were higher in fast- compared slow-growing chickens. The pectoralis major pH (6.18 vs. 5.83; $P<0.001$) and cooking losses (27.7% vs. 23.1%; $P<0.001$) were higher in fast- compared to slow-growing chickens. On the contrary lightness index (47.9 vs. 49.3; $P<0.001$), redness (0.18 vs. 0.58; $P<0.001$), and yellowness (9.79 vs. 11.3; $P<0.001$) were lower in former compared to the latter chickens. Overall, live HI larvae enrichment did not affect carcass traits or meat rheological traits ($P>0.05$). In conclusion, genotype had a significant impact on productive results, and carcass and meat quality traits, where both slow-and fast-growing genotypes showed wooden breasts, even if at a lower extent in the latter ones. This emphasises the key role of genetic background in determining growth performance and meat quality in broiler chickens. This work was co-funded by European Union's Horizon Europe Project 101136346 EUPAHW (SOA13, Task 2.3; SOA17, Task 1.4).

From tissue to transcriptome: Exploring differential gene expression in porcine scrotal hernia using a QuantSeq approach

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Scrotal hernia (SH) is a common congenital disorder in pigs and is characterized by the protrusion of abdominal contents into the scrotum, creating a visible bulge. Affected boars have an increased mortality risk due to intestinal strangulation, infections, and potentially fatal complications at castration. Beyond its effects on health and survival, SH also leads to considerable economic losses through reduced growth performance, increased veterinary care, and penalties at slaughter for intact boars, making it a substantial challenge for pig production. Despite its impact, the genetic basis and molecular mechanisms underlying scrotal hernia remain largely unknown. In this study, gene expression differences associated with SH were investigated using QuantSeq 3′ mRNA sequencing. As SH is closely linked to testicular descent and the anatomy of the inguinal canal, structures from the scrotal region were sampled, including the tissues composing the deep inguinal ring, the cremaster muscle, and the testis. These tissues were sampled bilaterally in both affected and unaffected piglets. In addition, the pituitary gland and hypothalamus were collected. Tissue samples were collected from affected (n = 13) and unaffected (n = 9) three-week-old crossbred piglets (Large White boar × Landrace sow) originating from four litters. Among the affected piglets, hernias were left-sided (n = 8), right-sided (n = 1), or bilateral (n = 4). All litters were sired by the same boar, and the dams were closely related as sisters or half-sisters. The relatively high incidence of SH within these related litters suggests a potential genetic contribution to the condition. RNA was extracted following standard protocols, after which QuantSeq libraries were prepared and sequenced. Resulting FASTQ files were mapped to the Sus scrofa 11.1 reference genome, and differential gene expression analysis was performed using DESeq2. Gene expression was compared between affected and unaffected animals in tissue samples collected from the deep inguinal ring region. QuantSeq analysis identified 140 differentially expressed genes ($\log2FC \geq |2|$; padj < 0.1), including 9 upregulated and 131 downregulated genes in deep inguinal ring tissue from affected animals compared with controls. The most significantly downregulated gene ($\log2FC = -3.69$, padj $= 8.55 \times 10$□6) functions as a transcriptional regulator of cell-type-specific differentiation pathways and has been implicated in the regulation of GnRH expression. Pathway analysis using Gene Set Enrichment Analysis and the Kyoto Encyclopedia of Genes and Genomes indicated enrichment of mitochondrial processes, particularly the electron transport chain and oxidative phosphorylation. Further analyses are ongoing, including differential gene expression analyses in additional tissues such as testis, cremaster muscle, pituitary, and hypothalamus, as well as analyses of genotype data to identify variants associated with scrotal hernia. Together, these findings suggest that altered transcriptional regulation and mitochondrial function may contribute to the pathophysiology of scrotal hernia and demonstrate the value of transcriptomic approaches for gaining molecular insight into this condition.

Biosecurity in challenging times: recognizing overlooked hygiene pathways in livestock production

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On-farm biosecurity has long been recognized as a cornerstone of disease prevention in livestock production. However, recent outbreaks of African swine fever and highly pathogenic avian influenza in Europe underline that biosecurity is not merely a theoretical concept but a practical necessity requiring continuous vigilance. While many preventive measures are well established, certain hygiene-related transmission pathways may still be underestimated in everyday farm management. Identifying and addressing these overlooked routes is therefore essential for improving disease prevention and strengthening farm resilience. This contribution summarizes findings from three recent studies investigating different hygiene-related risk pathways in livestock production systems. Airborne transmission was examined in a field study evaluating modified air-filter modules in a naturally ventilated turkey barn. Filtration reduced airborne particles and microorganisms by approximately 94 to 96%, and pathogens such as Ornithobacterium rhinotracheale were detected before but not after filtration, indicating the potential of air filtration as an effective biosecurity barrier. In another study, environmental persistence of pathogens was assessed through systematic sampling in broiler houses across several production cycles. Enterococcus cecorum was repeatedly detected in environmental samples, with increasing detection towards the end of the production period and occasional persistence even after cleaning and disinfection. These findings highlight the role of environmental reservoirs and illustrate the limitations of routine hygiene procedures in fully eliminating pathogens. A third study analysed drinking water quality on pig and poultry farms in Northwest Germany over a ten-year period. A considerable proportion of samples exceeded recommended guideline values, with 58.0% exceeding chemical limits and 47.4% exceeding microbiological limits, indicating that drinking water systems may represent an underestimated hygiene risk requiring improved monitoring and management. Taken together, these findings demonstrate that biosecurity challenges arise from multiple and sometimes underestimated pathways, including air, farm environment and water systems. Effective prevention strategies must therefore address these routes simultaneously and combine technical solutions with consistent hygiene management. In times of increasing disease pressure, strengthening animal hygiene and biosecurity is essential not only for farmers but also for veterinarians, advisors and scientists visiting farms, who all share responsibility for preventing pathogen transmission.

Live animal transport and disease spread: the public health case for meat and carcass trade

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Live animal transport is a significant, yet often under-recognised, contributor to the spread of infectious diseases transmission, with implications for animal health, public health, antimicrobial resistance (AMR), and livestock management. This presentation synthesizes evidence from peer-reviewed studies, EFSA reports, and outbreak analyses to examine the relationships between live animal transport, stress-induced disease susceptibility, and pathogen spread, and to evaluate the potential impact of alternative trade modalities, such as meat and carcass transport, on these risk pathways. Transport exposes animals to multiple stressors—including feed and water deprivation, confinement, thermal stress, and mixing with animals from different farms—which impair immune function and elevate disease susceptibility. In unweaned calves, transport coincides with the "immunity gap," when maternal antibodies decline before the calf's own immune system is fully developed, amplifying vulnerability. Empirical studies link transport stress with increased incidence of respiratory and enteric diseases, as well as higher mortality. Mixing animals during transport further amplifies pathogen transmission, illustrating the complex interplay between animal management, pathogen dynamics, and human exposure—core components of the One Health framework. Transport stress also has implications for AMR. Increased disease incidence during transport often necessitates antimicrobial treatment: according to a Canadian study on unweaned calves, reported treatment prevalence can reach 60–80%, reflecting substantial antimicrobial exposure. EFSA's scientific opinion on animal welfare during transport identifies handling practices as key drivers of pathogen dissemination, reinforcing the One Health connection between animal welfare, disease spread, and human health risks. From a One Health perspective, alternative trade strategies—particularly shifting to meat and carcass transport—may reduce these risks. Unlike live animal transport, carcass transport removes many direct pathways for pathogen amplification. Proper chilling and handling in meat supply chains reduces bacterial growth and limits viral transmission potential. European case studies illustrate the vulnerabilities of live animal trade: disruptions from disease alerts, such as foot-and-mouth disease outbreaks, and reconsideration of large-scale calf imports highlight the biosecurity challenges posed by live transport. This presentation highlights that reducing long-distance live animal transport could support animal welfare, mitigate disease spread, and potentially reduce the need for therapeutic antimicrobial use. Through a One Health lens, the findings indicate that prioritizing meat and carcass trade can strengthen connections between animal health, public health, and sustainable livestock management, underscoring how transport practices influence the health of animals, humans, and ecosystems.

Toward oral African swine fever vaccines: Harmonized evaluation and strategy optimization

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Keywords: African swine fever virus, live-attenuated vaccine candidates, oral administration, bait vaccination, harmonized infection models The African swine fever virus (ASFV) causes African swine fever, a severe disease with hemorrhagic-like fever and high mortality in suid species. African swine fever has developed into a global panzootic, affecting wild boar populations and the pig industry. Despite extensive biosafety measures and surveillance programs, ASFV control remains challenging. Eurasian wild boar act both as a reservoir of infection and as a species at risk for virus spread. Vaccination could serve as a crucial tool for containment, yet effective immunization of wild boar is only feasible via oral administration through baits. Although several live-attenuated vaccine candidates are promising, no widely approved vaccine is yet available for field use, and comprehensive data on oral ASFV vaccination strategies remain limited. Within an EU-funded research consortium, a harmonized multi-center study was conducted to standardize methods and readouts, and to compare three oral vaccine candidates. Therefore, domestic pigs were orally immunized with one of the three live-attenuated strains “ASFV-G-ΔI177L”, “ASFV-G-ΔMGF”, and “ASFV-G-Δ9GL/ΔUK”, followed by a challenge with the highly virulent ASFV strain “Armenia08”. Clinical signs, viral replication, and serological responses were monitored. Vaccination was well tolerated but general response rates were low. The best-performing candidate, “ASFV-G-ΔI177L”, achieved seroconversion in 20% of animals, with all responders fully protected. Building on these results, “ASFV-G-ΔI177L” was further tested in an optimization study using increased inoculation doses, adjuvant supplementation, and modified bait formulations. A ten-fold increase in vaccine dose raised seroconversion to 80%, while combining higher doses with walnut shell-shred baits or adjuvants achieved rates of 40%. The findings underscore the protective potential of the oral vaccine candidate and highlight the need for continued refinement of vaccine formulations and delivery systems to achieve an efficacious oral ASFV vaccine.

Staphylococcus aureus at the Wildlife–Livestock Interface: Occurrence and Genomic Characterization in Northern German Production Systems

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Introduction: Wildlife plays an important role in the maintenance and spread of endemic as well as emerging infectious diseases. The outbreak and continued spread of epizooties in Germany have highlighted the risks and challenges associated with disease control at the wildlife–livestock interface. At the same time, livestock production systems are becoming increasingly diverse, with growing interest in less intensive and more extensive husbandry systems. This development raises the question of which biosecurity measures, considering the structural characteristics of farms, are effective in preventing the introduction and spread of infectious pathogens. The aim of this study was to investigate the occurrence and genetic characteristics of staphylococci as microbiologic indicators, with particular emphasis on methicillin-resistant Staphylococcus aureus (MRSA), in huntable wildlife species and livestock sharing the same habitat. Material and Methods: Three locations in livestock-dense regions of northern Germany were selected for the investigation. A total of 14 farms were included, representing cattle, sheep, swine, and poultry production systems. Cattle farms included both intensive and extensive management systems. Sampling was conducted twice on each farm and throughout one hunting season for wildlife. Nasal swabs were collected from cattle and sheep, while boot swabs were used in intensive pig and laying hen production systems. Wildlife samples consisted of nasal swabs taken immediately after hunting. Recovered isolates were further subjected to in-depth characterization, including whole-genome sequencing. Results: In total, 127 wild animals were sampled, including roe deer (n = 59), wild boar (n = 23), fallow deer (n = 21), and European hare (n = 24). Although MRSA isolates were obtained from livestock or farm environments, no wildlife samples tested positive for MRSA. However, S. aureus was detected in several wildlife species, including roe deer (n = 24), wild boar (n = 11), fallow deer (n = 11), and European hare (n = 13). Genomic characterization revealed various spa types with only a few shared genotypes between wildlife and livestock. Discussion: The results do not allow a direct conclusion that extensive livestock systems facilitate the circulation of S. aureus between wildlife and livestock. However, some examples suggest a potentially increased risk of exchange in such systems. The intensity and duration of contact between wildlife and livestock may play an important role in shaping transmission dynamics. These findings contribute to a better understanding of pathogen circulation in shared habitats and support the development of targeted biosecurity measures in diverse livestock production systems.

Identifying the immunocompetence markers at weaning to predict vaccine response and persistence in pigs

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Identifying biomarkers of immunocompetence that predict vaccine response is crucial for preventing diseases, reducing antibiotic use, and enhancing vaccine efficacy in pigs. In this study, we aimed to identify immune markers measured at weaning (28 days of age) that could predict long-term antibody response and their persistence following vaccination against Mycoplasma hyopneumoniae (MH) and Influenza A virus (IAV) in pigs. We also investigated whether there were common predictive markers between the two vaccines. A cohort of 400 Large White pigs was vaccinated against MH (n=200) and IAV (n=200) at weaning and received a booster 21 days later. Vaccine responses were evaluated by measuring serum-specific antibody (Ab) levels against the pathogen at 28-, 35-, and 118-days post-vaccination (dpv). For each vaccine, the long-term response was classified as positive or negative based on antibody titre at 118 dpv. Antibody persistence was determined by calculating the decline from the peak titre (at 28 or 35 dpv) to the titre at 118 dpv, and animals were categorized as persistent or non-persistent. At weaning, a total of 58 key immune traits, including blood cell composition, hematocrit, plasma coloration, immunoglobulins, natural antibodies, electrophoretic protein profiles, enzymatic activities, C-reactive protein (CRP), and vitamin D, were measured to identify potential predictors of vaccine response. To evaluate predictive performance, we first applied sparse Partial Least Squares Discriminant Analysis (sPLS-DA) to identify the most informative markers using a tuning procedure. The markers selected through this process were subsequently used to build the final PLS-DA classification models. For MH vaccination, PLS-DA models achieved cross-validated accuracies of around 67.1% (AUC = 0.74) for long-term response and 66.6% (AUC = 0.71) for persistence classification. For IAV vaccination, the models reached accuracies of 66.4% (AUC = 0.71) for long-term response and 64.1% (AUC = 0.68) for persistence. Analysis of shared predictors indicated partially overlapping immune markers across vaccines. For long-term classification, mean corpuscular hemoglobin concentration (MCHC) and neutrophil percentage were consistently associated with a negative response in both MH and IAV. In contrast, hematocrit, plateletcrit (PCT), platelet count, and natural antibodies were consistently associated with positive responses. CRP exhibited opposite directional associations between vaccines. For persistent classification, vitamin D, alpha1 protein concentration, platelet count, and PCT were consistently associated with persistent responses across both vaccines, whereas MCHC and spectral absorbance traits were consistently linked to non-persistent responses. These findings indicate that early-life immuno-hematological traits contribute to variation in long-term vaccine responsiveness and antibody persistence. Incorporating these biomarkers into breeding or management strategies may enhance vaccine efficacy and improve overall herd health and resilience.

Semen storage at 5 °C enables omission of antibiotics in artificial insemination of pigs under field conditions

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Pigs are the only species in which extended semen doses for artificial insemination (AI) are conventionally stored at 16-18 °C and cryopreservation remains not routinely available. To protect semen from potential harmful bacterial growth at this temperature, antibiotics are added to the semen extender. However, this prophylactic use increases the risk of antimicrobial resistance. With the development of novel cooling methods, despite the chilling sensitivity of boar spermatozoa, it is now possible to preserve semen in a liquid state while minimizing antibiotic use. The aim of this study was to test the semen storage at 5 °C in vitro and in vivo under field conditions as a strategy to reduce antibiotics in semen extenders. For in vitro testing, ejaculates from nine boars were split, diluted in Androstar® Premium semen extender (Minitüb GmbH) and stored at 5 °C or at 17 °C as a control. In a similar setup, the field trial used semen doses from a commercial AI-center to inseminate sows in two customer farms. Monitoring of bacterial count in antibiotic-free semen stored for 144 h revealed that storage at 5 °C effectively inhibits bacterial growth. Cold-stored samples spiked with drug-resistant, spermicidal Serratia marcescens kept bacterial counts below ~10^3 CFU/mL and maintained sperm quality up to 144 h of storage. In contrast, control samples stored at 17 °C showed significantly higher ($p < 0.05$) bacterial loads of > 10^{11} CFU/mL concomitantly with sperm agglutinations and loss of motility. Testing a population of 20 boars in an AI center, each with four consecutive ejaculates, revealed broad eligibility (> 90 % of the boars) for 5 °C semen storage based on sperm motility and membrane integrity. In semen doses of 10 boars used for AI, sperm motility in the 5 °C group was slightly lower than in control (< 5 %), which was significant in a two-way ANOVA ($p = 0.01$). All 5 °C samples met the standard requirements for AI defined by the German Livestock Association (BRS e.V.). The fertility of a total of 579 sows across two different farms was not affected by semen storage temperature, with no differences observed in pregnancy rates ($p = 0.29$, χ^2 test) or in litter size ($p = 0.16$, ANOVA). In conclusion, combining the results of in vitro experiments on bacterial growth and sperm quality with fertility data on customer farms, our findings demonstrate that storage of boar semen at 5 °C is a sperm-friendly and biosafe preservation method suitable for broad boar population. The novel 5 °C semen storage concept provides a sustainable alternative in pig reproduction by enabling the omission of antibiotics in semen extender while maintaining high fertility under field conditions. Supported by the Federal Ministry of Food, Agriculture and Regional Identity (BMLEH) based on a decision of the Parliament of the Federal Republic of Germany and by the Association for Bioeconomy Research (FBF e.V.) (281DT01B21).

Exploration of somatic cell counts in sow colostrum

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Sows can produce 3 to 5kg of colostrum and reach milk yields of above 10kg a day. In a pilot study we studied correlations between somatic cell counts (SCC) and key quality and health indicators. We hypothesized that SCC in sow colostrum would be positively correlated with the presence of pathogenic bacteria. We also hypothesized that SCC would be negatively correlated with colostrum quality. The study was conducted in accordance with UK Animal (Scientific Procedures) Act 1986 legislation. 36 sows (landrace x large white) housed in conventional individual farrowing crates/pens and managed as per commercial standards were used. On the farrowing day, colostrum samples from each sow were collected within 3 h of the onset of farrowing pooling from several teats (teats were cleaned before collection and the first stream of colostrum discarded). Samples were used for bacteriology examination (e.g., Staphylococcus spp., Staphylococcus Aureus, Enterococcus/Streptococcus, Total viable count of mesophilic organisms (TVC), and Total Lactic Acid bacteria (LAB)) and for composition analysis using a Milkoscan Model FT120 (Foss Electric), immunoglobulin G (IgG) content using digital Brix refractometer, and SCC determination using a fluoroscopy based SCC counter (Lactoscan SCC, Milkotronic Ltd.). The first 10 piglets born from each sow were weighed at birth and at 24h (used to calculate colostrum intake as per Devillers et al., 2004) and blood sampling attempted at day 5 of life to measure IgG levels. Statistical analyses were performed using Genstat 24th edition (Version 24.2). Descriptive statistics were calculated for all study variables. A correlation matrix was constructed to evaluate the relationships between SCC and bacteriological parameters, colostrum quality parameters, and IgG concentration in colostrum and blood. Correlation coefficients were estimated using Pearson's method and statistical significance was set at $P < 0.05$. SCC counter readings (x1000) averaged 1,344, range 148 to 14,004. SCC readings were positively correlated with cellular count from the lab/Milkoscan ($P=0.028$, $r=0.42$). Among bacteriology parameters, SCC was only correlated with Enterococcus/Streptococcus ($P=0.003$, $r=-0.54$). SCC readings were negatively correlated with total colostrum intake ($P=0.055$, $r=-0.37$). No further correlations were found between SCC and the recorded parameters. Cellular count from lab/Milkoscan was correlated with TVC ($P=0.004$, $r=0.56$) and LAB ($P=0.032$, $r=0.43$). Staphylococcus aureus was negatively correlated with protein ($P=0.053$, $r=-0.35$), casein B ($P=0.034$, $r=-0.38$), fat ($P=0.039$, $r=-0.37$) and solids-non-fat content ($P=0.011$, $r=-0.44$). Staphylococcus spp. was negatively correlated with fat ($P=0.093$, $r=-0.31$). Piglet serum IgG was correlated with digital Brix refractometer results ($P=0.023$, $r=0.41$). Despite both cellular determinations (i.e., SCC count and cellular count) were highly correlated and in agreement, only results obtained from lab/Milkoscan were positively correlated with bacterial growth (TVC and LAB). Fluoroscopy-based SCC did not yield significant correlations with classical bacteriology though it was negatively correlated with colostrum intake. However, no relationship was found between SCC and serum IgG levels in pigs. Further research is needed to validate SCC determination for sows. Nonetheless, the presence of pathogenic bacteria (i.e., Staphilococcus aureus) was negatively correlated with colostrum quality. Our work has identified links between the presence of bacteria in sow colostrum and its quality, with potential implications for piglet health. Further research is needed to better understand the implications such links could have for immunity transfer and piglet health. References: Devillers et al., 2004. Anim. Sci. 78: 305-313

Elevated blood glucose in late gestation is associated with increased mortality, particularly in gilts

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Low-vitality newborn piglets are at increased risk of being overlain and are less able to access colostrum, substantially reducing their likelihood of survival. These piglets require additional care in the immediate postnatal period. There is increasing evidence that gestational diabetes in sows may lead to perinatal asphyxia which is hypothesised to cause foetal hyperinsulinemia and increased oxygen demand which in turn can lead to chronic hypoxia and vulnerability to intrapartum asphyxia, neonatal hypoglycaemia and mortality. Therefore, the aim of the present study was to determine any relationships between maternal indices of gestational diabetes and piglet growth and survival. Gestating sows (Large white x Landrace) x Danish duroc) were housed under commercial conditions in a dynamic herd. At approximately day 109 of gestation, 145 sows and gilts were weighed, body condition scored and had backfat measurement taken using an ultrasound scanner. The following day sows had a single blood sample taken from the mammary vein for analyses of glucose and insulin concentrations. The average plasma glucose concentration was 4.94±0.163 (mean±SEM) mM with a range of 3.37 to 17.3 mM while plasma insulin concentrations averaged 36.4±7.07 IU/L with a range of 0.03 to 375 IU/L. Regression analyses of sow and litter performance against actual glucose and insulin parameters failed to reveal any significant correlations perhaps because of the very large variations in range and skewness of the data. Therefore, plasma glucose data were categorised into quintiles with 1 being the lowest quintile and 5 the highest quintile and correlation analyses performed. Plasma glucose quintile was positively associated with number born alive and measures of piglet mortality. For example, the number of deaths in the first week ($r=0.16$, $p=0.031$) as well as birth ($r=0.18$, $p=0.016$) and reared litter mortality ($r=0.17$, $p=0.023$) all increased with increasing glucose quintile. Therefore, birth litter mortality increased linearly from 7% to 12% with increasing plasma glucose quintile. Interestingly, this response was most pronounced in gilts where birth litter mortality increased linearly from 0 to 23.5% with increasing glucose concentrations ($r=0.55$, $p=0.016$) whereas mortality was relatively constant for older parities ($r=0.076$, $p=0.41$). There were no significant relationships with plasma insulin concentrations. These data demonstrate that there is a wide variation in both plasma glucose and insulin concentrations in late gestating sows and that elevated plasma glucose in late gestation is associated with increased piglet mortality, particularly in gilts.

Aligning veterinary recommendations, farmer adoption, and costs of preventive measures for production diseases in Danish dairy herds

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Dairy cattle productivity has increased over the last 50 years. This brings each cow under more stress, increasing her susceptibility to disease. Diseases in dairy cows represent inefficiencies of economic and environmental impacts due to losses in feed intake, productivity, and longevity. Total costs of disease can be understood as the sum of failure cost (FC) and preventive cost (PC). Farmers' decisions are in part driven by economic incentives, but they tend to underestimate FC, leading to underuse of preventive measures (PM) from a financial perspective. We examined the PM and PC associated with lameness, mastitis and metabolic diseases. We aimed to obtain lists of PM ranked by veterinarians and farmers, to find whether the respondents in each group agreed on the ranking, and whether the farmers pay more for PM ranked higher based on PC. We surveyed 147 veterinarians, followed by interviews with 22 dairy farmers. The survey included 156 PM-disease complex combinations. For each combination, the veterinarians assessed the PM effect on the disease complex in 6 categories: Don't know, None, Some, Great, Conclusive, or Indirect. Farmers assessed the top 3 PM implemented on their farm within each disease complex. They provided an estimate of investments, labour time (LT) and operational cost (OC) of each PM. The PM assessed by the farmers were extracted from the interviews and treated such that measures with no information or on-farm implementation were excluded. Preventive measures where all of the investments, LT and OC could be split into separate measures were split, and separate measures that included the same costs were merged. The interrater agreement among veterinarians and farmers was found using the proportional overlap method. To compare PC and effect for each PM, the categories "None", "Some", "Great" and "Conclusive" were treated as a Likert scale. To estimate the PC of each measure, cost factors were calculated in EUR per cow per year. Investment costs were calculated with estimates of the investments' lifetimes and labour costs using the collective agreement wage for 2023. PC for each PM is the sum of IC, LC and OC scaled by the degree to which the PM was used for the disease complex and only calculated if all four values were available. Spearman's rank correlation was calculated for PC and effect, and LT and effect, for each disease complex. The ranked PM-lists only included PM's assessed as having "Great" or "Conclusive" effect. The veterinarians' ranked list included 141 PM, and the farmers' 43 PM. For lameness, mastitis and metabolic disease, the PM ranked highest by the veterinarians were systematic claw trimming, proper maintenance and settings of the milking system and rapid correction of diet on signs of problems. For the farmers, it was systematic claw trimming, having clean bedding and phase-feeding of dry cows. The interrater agreement for all PM ranked by veterinarians was $\kappa=0.22\pm0.0024$, for the farmers $\kappa=0.0091\pm0.021$. All Spearman's rank correlations were lower than 0.2, and none of the correlations differed significantly from 0. We concluded that interrater agreement within veterinarians' and farmers' assessments of PM was low. The farmers had different frames of reference for assessing the PM efficiency as they manage herds with different characteristics. There was no correlation between PC and the farmers' assessment of the effect on incidence for either lameness, mastitis, or metabolic disease. Farmers do not pay more for PM ranked higher based on the PC. Use of better decision support tools could benefit farmers in choosing the right PM's.

Mastitis in sows and its implications for colostrum quality

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Mastitis in sows is frequently mentioned but has received little research attention in the past years. In a pilot study, we assessed the presence of mastitis in sows and we hypothesized that mastitis would be linked to the presence of pathogenic bacteria and to colostrum quality. We also hypothesized that mastitis would negatively effect piglet pre-weaning growth. The study was conducted at AFBI pig research farm in accordance with UK Animal (Scientific Procedures) Act 1986 legislation. 36 sows (landrace x large white) and their piglets (only the first 10 piglets born were monitored) housed in conventional individual farrowing crates/pens were used. On the farrowing day, sows were assessed for mastitis with a score adapted from Rosengart et al. (2021) based on formation/regression, redness, consistency, presence of nodes and painfulness of the udder. Colostrum samples from each sow were collected within 3 h after the start of farrowing, pooling from several teats (teats were cleaned before collection and the first stream of colostrum discarded). Colostrum was analysed for composition (protein, fat, lactose, and total solids) using a Milkoscan Model FT120 (Foss Electric) and for classical bacteriology: colony formation units (CFU/ml) of Staphylococcus spp., Staphylococcus aureus, Enterococcus/Streptococcus, total viable count of mesophilic organisms (TVC) and total lactic acid bacteria (LAB). In addition, presence of somatic cell counts (SCC) was determined using a fluoroscopy-based SCC counter (Lactoscan SCC, Milkotronic Ltd.). Piglets from sows were weighed at 24h after birth and at weaning (28 days), and blood sampling was attempted on day 5 of life for immunoglobulin G (IgG) serum determinations. Statistical analyses were conducted using Genstat (24th edition, version 24.1). Data were analysed using separate linear mixed models fitted by Restricted Maximum Likelihood (REML) to evaluate the association between sow mastitis score, colostrum composition and piglet performance. All variables included parity and sow body weight as covariate when significant, and piglet body weight at weaning also included piglet body weight at 24 h as covariate. Effects were considered significant at $P < 0.05$. Total mastitis score ranged from 0 to 5, though sows were finally classified into: 0, 1, 2 and ≥3. Nonetheless, 22% of the sows (n=8) had a score of ≥3 and only 19.4% had a score of 0 (n=7). There were 10 sows with a score of 1 and 11 sows with a score of 2. No purulent discharge or 'no feeding' were documented, only 2 sows showed painful reaction to udder touch and all sows completed lactation. Mastitis score had no significant effect on the presence of bacteria ($P>0.05$), though it is worth mention that sows with score ≥3 had numerically almost twice the amount of TVC (0.644 and 1.188 CFU/ml, respectively) and four times the amount of Enterococcus/Streptococcus (0.291 and 1.416 CFU/ml, respectively) than sows with score = 0. Similarly, mastitis score had no significant effect on colostrum composition. Finally, piglet weight at 24h (av. 1.38 ± 0.134kg) and weaning (av. 8.24 ± 0.618kg) did not differ among groups ($P>0.05$, respectively). Presence of mastitis was not linked to the presence of bacteria in sow colostrum. Similarly, presence of mastitis did not affect piglet body weight at weaning, although our data was limited to the first 10 piglets born as opposed to the whole litter. The lack of severe cases of mastitis could explain the lack of differences observed. Nonetheless, a high proportion of sows had some degree of mastitis. Further research with increased number of sows would be necessary to properly quantify mastitis incidence and its implications when presented in more sever forms. References: Rosengart et al., 2021. Agriculture 11, 1013

Assessment of the economic impact of swine influenza on Japanese swine commercial farms

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Background: Swine influenza is a respiratory disease caused by influenza A viruses of swine (IAV-S), which is one of the major respiratory pathogens affecting pigs in the world. The virus typically spreads rapidly through a susceptible population due to its high transmissibility and the short incubation period that precedes virus shedding. Infection results in clinical signs of fever and coughing, which leads to decreasing productivity and substantial financial losses. However, no study has yet quantified the economic losses of IAV-S in Japanese swine commercial farms. Therefore, the objective was to quantify the economic impact of IAV-S infection on Japanese swine commercial farms using a stochastic simulation model. Methods: This study collected data from 11 farrow-to-finish commercial farms in Japan. Active surveillance was performed in July 2024, October 2024, and January 2025 to collect nasal swab samples that were randomly collected from 30 weaned pigs by veterinarians on each farm. The samples were examined for the presence of IAV-S by real-time RT-PCR. Farms were classified into two groups based on the frequency of IAV-S detection. Of the 11 farms, five farms had at least one IAV-S positive sample in all three surveillance, defined as high IAV-S farms, six farms had at least one IAV-S positive sample in zero, one or two surveillance, defined as low IAV-S farms. In this study, the economic impact is expressed in annual margin over feed cost per sow (MOFC), which was estimated by subtracting annual feed cost of marketed pigs per sow from annual revenue of marketed pigs per sow. A Monte Carlo stochastic simulation modeling with @RISK software was used to estimate MOFC under two scenarios: IAV-S high and IAV-S low farms. For each IAV-S status scenario, probability distributions were fitted to the observed data using @RISK's distribution fitting functions. Each scenario was simulated using 10,000 Monte Carlo iterations to generate the distribution of MOFC. Economic unit used in this analysis was Euro (€). In January 2026, the exchange rate is such that €1 equals 185 JPY. Results: The simulation input items based on farm data were post-weaning mortality, vaccine and medication costs per market pig, and carcass based feed conversion ratio. Based on these items and the references data, the simulation output results are as follows. Mean±SD and associated 90% confidence interval (CI, 5 to 95%) of MOFC under the IAV-S low scenario was €3,267±205 and €2,866 to 3,443, whereas those under IAV-S high scenario was €2,774±336 and €2,170 to 3,194. Under IAV-S low scenario, annual revenue of marketed pigs per sow was €6,085±78, feed cost of marketed pigs per sow was €2,817±204, and the number of market pig per sow was 26.5±0.3. Under IAV-S high scenario, these items were €5,518±304, €2,742±338, and 24.1±1.3 pigs, respectively. Discussion & conclusion: This study showed that IAV-S infection damaged economic performance, as IAV-S low farms had higher MOFC than IAV-S high farms. This is because IAV-S low farms had higher annual revenue of marketed pigs per sow, while feed cost of marketed pigs per sow were similar for both farms. Specifically, the lower revenue in IAV-S high farms is attributed to a reduction in the number of market pigs per sow, resulting from higher post-weaning mortality. In conclusion, result from model indicate that IAV-S infection damaged the profitability of Japanese swine commercial farms. Therefore, it is important to prevent IAV-S from spreading and not to ignore its impact on farm management.

Analysis of factors associated with preweaning mortality and cause-specific mortality

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Background: Preweaning mortality in pig production is an important issue from both productivity and animal welfare perspectives. In recent years, increasing litter size associated with genetic improvement may elevate mortality risk through a higher proportion of low birth-weight piglets. Preweaning mortality is a multifactorial problem influenced by maternal characteristics, litter size, birth weight, management-related factors, and environmental conditions. However, the determinants of cause-specific mortality under commercial production conditions remain insufficiently characterized. This study analyzed factors associated with preweaning and cause-specific mortality using production records from commercial swine farms in Japan. Material and Methods: Farrowing records (n = 22,418) collected between 2012 and 2015 from four commercial farrow-to-finish farms in Japan were analyzed. Three mortality outcomes—preweaning mortality, crushing mortality, and weakness-related mortality—were analyzed using separate binomial generalized linear mixed models with a logit link function, including sow ID as a random effect. Preweaning mortality was defined as the number of piglets that died during lactation divided by the number of piglets at the start of lactation after cross-fostering. Explanatory variables included parity, gestation length (GL), pigs born alive (PBA), piglet birth weight, cross-fostering, lactation length (LL), and farrowing season. Final models were selected using backward elimination, and statistical significance was declared at $P < 0.05$. Results: The mean ± SD rates for overall preweaning, crushing, and weakness-related mortality were 9.1±12.3%, 4.1±7.6%, and 2.1±5.9%, respectively. All mortality outcomes were significantly associated with parity, GL, PBA, piglet birth weight, cross-fostering, and farrowing season ($P < 0.05$), but not with LL. Higher rates for all mortality outcomes were observed in litters from high-parity sows, litters with a high PBA, litters with a low birth weight, litters that received fostered piglets, and sows that farrowed between April and June ($P < 0.05$). Regarding GL, a short GL was associated with higher overall and crushing mortality, whereas a long GL was associated with higher weakness-related mortality ($P < 0.05$). Discussion & conclusion: Using large-scale commercial production data, this study identified key determinants of preweaning and cause-specific mortality on Japanese swine farms. Litters with a lower PBA showed a reduced mortality risk, suggesting that larger litters may increase mortality through intensified competition among piglets and greater variability in birth weight. Low birth weight was strongly associated with an increased mortality risk, indicating that birth weight is a fundamental determinant of survival during the lactation period. Litters from high-parity sows may have greater variability in birth weight, which results in higher mortality. The higher mortality in litters that received fostered piglets is likely due to the addition of inactive piglets. Overall, targeted management of these high-risk litters may contribute to reducing preweaning mortality. This study provides quantitative evidence of the multifactorial determinants of preweaning mortality in commercial swine production.

Tricks of the trade: understanding cattle purchasing in the context of an endemic statutory disease and associated control efforts

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Bovine Tuberculosis (bTB) is an endemic infectious disease of cattle in Ireland. Statutory eradication efforts began in the 1950s, but despite considerable progress, eradication has not been achieved. The movement of undetected infected cattle through trade into previously uninfected herds has been identified as an important transmission route between herds. Herds recently released from movement restrictions following the removal of test-positive animals have been shown to be at increased risk of subsequent breakdowns (i.e., detection of bTB within a herd). The limited sensitivity of the intradermal (tuberculin) diagnostic test results in some infected cattle remaining undetected. These animals may continue to spread bTB among herd mates. If such an animal is unintentionally sold, infection may be introduced into the buyer's herd. Thus, purchasing cattle is a known risk factor for disease incursion. Risk-informed and risk-based trading (RBT) has been suggested as a potential mitigation measure, facilitating the informed and responsible trade of cattle. However, as this approach hinges on farmer compliance, farmer acceptability is crucial to its success. Therefore, understanding how bTB is perceived in the context of the broader stressors experienced by farmers may provide policymakers with insights into how/why farmer buying decisions occur, and whether future policies would be accepted or resisted. Irish cattle farmers were recruited using purposive quota and snowball sampling within a qualitative social science framework. Ten dairy and eleven beef suckler farmers were interviewed. Interviews were audio-recorded, anonymised and transcribed. Reflective thematic analysis, following Braun and Clark's six-phase framework, was used to analyse the transcripts. Themes were discussed and refined with a second coder for improved validity. Interviews lasted an average of 19 minutes (range: 10-62). Four themes emerged: (1) the multifaceted nature of cattle purchasing, balancing desirable animal traits, affordability, and the risk of various infectious diseases; (2) the influence of the presence of bTB on purchasing decisions; (3) how farmers' lived experiences shape farmer welfare and influence perceptions of bTB control; and (4) hopes, fears, and adjustments of risk-based trading interventions necessary for improved acceptance by farmers. Cattle purchasing decisions are shaped by farmers' preferences, practical needs, finances, social influences, experience, and the broader political context. Understanding how bTB risk fits among these factors can help explain compliance with bTB risk–informed recommendations. Many farmers want more disease-risk information when purchasing, but support for RBT policies varied widely.

Genetic population structure and genomic associations with coat colour and conformation traits in the endangered Schleswig cold blood horse

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With an active breeding population of less than 200 animals, the Schleswig coldblood horse is listed as an endangered livestock breed. As part of an ongoing project to support breed conservation while preserving genetic diversity within the closed studbook, the aim of this study was to analyse the variation within the population based on genomic data. Furthermore, available phenotypic information on body measures and owner-declared coat colour were used for a first screening for indications of candidate genes and chromosomal regions for relevant phenotypic traits as defined in the breeding program. Based on collected samples of Schleswig coldblood horses and single nucleotide polymorphism (SNP) genotyping with the Equine80select genotyping array (Illumina), a dataset of 398 horses with genotypes of 81,974 SNP markers was analysed. Body measures height at withers (WH), cannon bone circumference (CBC) and chest circumference (CC) from studbook registrations as well as genotypic and pedigree data were provided by the studbooks via IT Solutions for Animal Production (vit). Information on coat colour was taken from owner questionnaires. After quality control 392 horses with 67,545 SNP marker genotypes remained in the study. The number of genotyped horses among those with phenotypes were 275 for coat colour, 170 for WH and CC, and 171 for CBC. The principal component analysis (PCA) of the genomic datasets using PLINK implied clustering of within-population genetic variation by popular sires. A genome wide association study (GWAS) was conducted with the BLUPF90 software for each phenotype trait using ssGBLUP with a random animal effect. GWAS revealed a region on ECA 3 strongly associated with base coat colour. The region contains the MC1R gene, which is known to affect coat colour. GWAS for CBC implied an involvement of the ZFAT gene on ECA 9, previously reported for its influence on conformation traits in Thoroughbred and Swiss Franches-Montagne horses. Furthermore, putative regions associated with WH and CC were located on ECA 1 and ECA 15, but not in the well described region of LCORL on ECA 3. The results contribute to the limited knowledge on the genome of coldblood horses and indicate challenges that may arise when working with data from small populations of rare breeds. Methods for characterization of small breeds, tools for optimal suggestions of matings to preserve genetic diversity within breed, and potential associations of chromosomal regions with phenotypic traits are subject to further ongoing research.

Genetic investigations of the roan coat color in Icelandic horses: Population analyses and candidate variants on ECA3

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Roan is a coat color phenotype characterized by an intermixture of white and pigmented hairs on the body, while head and distal limbs remain unaffected. In several livestock species, roan is associated with variants in KIT or KITLG and may show pleiotropic effects, including sub-lethality or reproductive disorders. In horses, roan has been suspected to be homozygous lethal, although the causative mutation is still unknown. Given the documented health implications in other species, elucidating the genetic basis of roan in horses is relevant not only for fundamental research in coat color genetics, but also for animal health and welfare, particularly regarding breeding regulations aiming to avoid hereditary defects (e.g.§11b German Animal Welfare Act), and for evidence-based breeding strategies. This project integrates complementary approaches to (i) assess the population status of roan in Icelandic horses and statistically evaluate mating outcomes with respect to the lethality hypothesis, and (ii) identify candidate variants on equine chromosome 3 (ECA3) associated with the phenotype. The Icelandic horse represents a closed population with documented pure breeding for over one thousand years and an explicit breeding objective to preserve a broad spectrum of coat colors, underlining the particular relevance of roan for this breed. Using data from WorldFengur, the official international studbook for Icelandic horses, descriptive statistical analyses were conducted. The total number of Icelandic horses currently registered as roan amounts to 3,232, corresponding to approximately 0.5% of the global population. Evaluation of roan × non-roan matings (n = 3,940) confirmed dominant inheritance. Analysis of roan × roan matings (n = 103) did not support the hypothesis of homozygous lethality, as offspring ratios were consistent with Mendelian expectations and viable homozygous individuals were identified. In a first molecular approach, all 21 exons of KIT and adjacent intronic regions were sequenced in 30 roan and 23 non-roan Icelandic horses. Several variants showed significant association with the roan phenotype; however, none could be confirmed as causative. Nevertheless, the localization of associated variants within and near KIT further supported the hypothesis that roan in Icelandic horses maps to the ECA3 region encompassing this gene. Subsequently, whole-genome sequencing was performed in two roan Icelandic horses (one heterozygous, one marker-tested homozygous). Variant filtering focused on the candidate region on ECA3 (78–82 Mb) and was complemented by sequence data from additional Icelandic horses and roan and non-roan individuals of multiple breeds. Two filtering strategies were applied: (i) identification of a common roan-associated variant across breeds and (ii) detection of an Icelandic-horse-specific variant, indicating genetic heterogeneity. Both approaches revealed promising candidate variants near KIT. A structural Indel, initially considered a potential marker for roan in Icelandic horses, could not be validated after targeted resequencing in an extended Icelandic horse sample set. In conclusion, the combined population-based and molecular analyses provide evidence against homozygous lethality of roan in Icelandic horses and support linkage of the phenotype to the KIT region on ECA3. The absence of a confirmed shared causative variant across breeds suggests genetic heterogeneity of roan in horses. These findings contribute to understanding equine pigmentation genetics and provide a scientific basis for responsible, welfare-oriented breeding decisions.

Towards a comprehensive screening approach for detecting gene doping in racehorses and sport horses by targeting common and intrinsic plasmid sequences

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The rapid evolution of biotechnology and gene therapy may unfortunately facilitate the emergence of gene doping. In horse racing and equestrian sports, this practice, defined as the use of genetic tools to modify the genome, its expression or its regulation, to enhance the individual's performance, is prohibited by the International Federation of Horseracing Authorities (IFHA) and the International Equestrian Federation (FEI). One of the main methods likely to be misused is the use of eukaryotic expression plasmids, as they are easy to produce, customisable, and capable of carrying a wide range of performance transgenes. [1-3]. Current detection methods are primarily based on the specific identification of transgene sequences, however if the sequence is unknown or modified, doping may go undetected [4-5]. The aim of this study was to overcome this limitation by developing an untargeted transgene screening approach based on plasmid detection. A database of 349 mammalian expression plasmids was compiled, enabling the identification, through in silico sequence analysis, of four consensus sequences: the ampicillin resistance gene (AmpR), the cytomegalovirus promoter (pCMV), and two unannotated plasmid backbone sequence located between functional element (named IF1 and IF2). Together these targets covered 100% of the plasmids in the database. A set of PCR hydrolysis probe assays targeting these sequences was developed and demonstrated excellent efficiency [97.8% – 100.5%], linearity ($R^2 > 0.99$) and robustness, as assessed by inter- and intra-day %CV, matrix effect and extraction yield in sample from 30 horses. By determining a screening cut-off value using statistical ROC curve analysis, the detection method was able to discriminate plasmid signals from background noise in 100% of the suspicious plasma samples down to 1,200 copies/mL. Following administration of the pcDNA3.1 plasmid containing AmpR, pCMV, IF1, and IF2 to a horse, all four targets remained detectable for up to 48 hours using qPCR and dPCR, demonstrating proof of concept in an in vivo application. To explore potential explanations following the observation of some amplification signals with very high Cq values in a few plasma samples not supplemented with plasmids, an inventory of circulating cell-free DNA (ccfDNA) in horses will be conducted using long-read sequencing on the PromethION 2 Solo benchtop device, before and after exercise performed by the animals. This will enable the characterization of endogenous circulating genetic material in horses. Overall, this work presents a sensitive, reliable, and transgene-independent method which strengthens plasmid doping detection, reinforces gene doping control, and enables a substantially improved characterisation of the genetic material circulating in the equine plasma matrix. [1] Kovac M et al., 'Gene Therapy Using Plasmid DNA Encoding VEGF164 and FGF2 Genes: A Novel Treatment of Naturally Occurring Tendinitis and Desmitis in Horses'. (2018) [2] Baoutina et al., 'Potential Use of Gene Transfer in Athletic Performance Enhancement'. (2007) [3] Haughan, Ortved, and Robinson, 'Administration and Detection of Gene Therapy in Horses'. (2023) [4] Wong et al., 'A Multiplex qPCR Assay for Transgenes Detection'. (2024) [5] Tozaki et al., 'Droplet Digital PCR Detection of the Erythropoietin Transgene from Horse Plasma and Urine for Gene-Doping Control'. (2023)

Wearable-based continuous monitoring and omics profiling of thermal and exercise resilience in Thoroughbred racehorses

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Horses are a valuable model to investigate resilience to combined heat and exercise stress. Heat load and exertion elicit systemic adaptations to maintain thermoregulation, but under strenuous conditions, these mechanisms may become insufficient, increasing maladaptation risk. This study aimed to characterize physiological and molecular responses to heat and exercise stress by integrating continuous wearable monitoring (Equimetre™), hematobiochemical parameters, and circulating microRNA (ci-miRNA) sequencing. Fifty-eight Thoroughbreds from one training centre (Rome, Italy) were monitored during trainer-defined workouts. Ambient temperature and humidity were recorded by Equimetre™ and horses were grouped as COLD <10°C (n=23), INT 10–20°C (thermoneutral; n=17) or HOT ≥20°C (n=18). GNSS/IMU locomotor metrics and ECG-based heart-rate (HR) and recovery indices were collected; rectal temperature was recorded post-exercise. Blood was sampled pre-exercise and 10 min post-exercise for complete blood count and lactate; serum small RNAs were sequenced (Illumina, 75-bp single-end). Group comparisons used ANOVA or Kruskal–Wallis ($p<0.05$). Reads were mapped to miRBase v22.1 and EquCab3; DESeq2 was used for differential expression (FDR<0.05, $|\log_2 FC|\geq 1$). Post-exercise lactate did not differ among environments (~10–11 mmol/L). Rectal temperature tended to be higher in HOT than INT (39.2±0.4 vs 38.8±0.5°C; p=0.065). Mean speed during fast work (Vmean) differed (p=0.015), being lower in HOT than INT, whereas distance, Vmax, stride variables and speed-at-target-HR indices were unchanged. Time >90% HRmax tended to increase in HOT, while HR at 15-min recovery was higher in INT than HOT (p=0.02) and COLD (p=0.04), suggesting delayed recovery when humidity was higher despite thermoneutral temperature. Post-exercise white blood cells (WBC), hemoglobin (HB) and hematocrit (HCT) were higher in HOT than COLD ($p\leq 0.04$), with higher mean cell volume (MCV) and increased monocytes, eosinophils and basophils, consistent with hemoconcentration and stress-related leukocyte mobilization. ci-miRNA differential expression emerged only post-exercise (HOT vs INT), identifying 15 miRNAs. miR-221/miR-326/miR-28 align with immune-cell activation and trafficking, supporting leukocyte shifts; miR-130a/miR-221 with endothelial remodeling and hypoxia signaling, consistent with higher cardiovascular strain and reduced speed efficiency; and miR-23a/miR-375 with metabolic–endocrine stress, coherent with heat-related homeostatic pressure and hemoconcentration. Together, these pathways suggest a coordinated inflammatory–vascular–metabolic response detectable immediately post-exercise. Overall, thermoneutral conditions supported the most favorable performance–physiology balance, whereas early-season warmth increased thermal and cardiovascular load and triggered a distinct post-exercise ci-miRNA signature. This integrated wearable–hematobiochemical–miRNA framework may help track acclimatization, tailor training intensity and support welfare by detecting early signs of maladaptation to thermal stress.

Mitochondrial and genomic factors in equine Recurrent Exertional Rhabdomyolysis

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Introduction: Recurrent Exertional Rhabdomyolysis (RER) is a muscle disorder affecting Standardbred horses, a breed selected for high-speed trotting performance. Affecting approximately 6% of the population[1], RER is characterized by exercise-induced muscle cramping that impairs locomotion. Previous studies[2] have shown moderate heritability in several breeds, including Standardbreds, with heritability estimates ranging between 0.39 and 0.49. Although transcriptomic alterations in metabolism and calcium homeostasis have been reported[3], the underlying genetic determinants of RER remain unknown. We hypothesized that RER has a complex polygenic determinism involving probably both nuclear and mitochondrial genetic variants. Material & Methods: Following ethical approval, 48 French Standardbred horses (25 affected by RER and 23 matched controls) were sampled. Inclusion criteria included regular training for trotting races, age >2 years, at least two confirmed RER episodes for affected horses, and no history of RER for controls. Blood (10 mL) was collected in EDTA tubes, and DNA was extracted and quality-controlled prior to sequencing. Whole-genome sequencing was performed using Illumina paired-end short-read technology (150 bp). Bioinformatic analyses included sequencing quality control, alignment to the EquCab3 reference genome, and nuclear variant calling using GATK4 HaplotypeCaller. Mitochondrial DNA (mtDNA) was analyzed separately using a shifted mitochondrial reference genome to account for mtDNA circularity and haploidy, with variant calling performed using Mutect2 in mitochondrial mode. A non-stringent minimum allele frequency threshold of 1% was applied to both nuclear and mitochondrial datasets to exclude singletons. Variants were annotated using snpEff, and Chi-square tests were used to compare variant frequencies between RER-affected and control horses. Results: Whole-genome sequencing depth averaged 21.7 ± 9.3X. After quality filtering, 98.4% of reads passed quality control, with all FastQC scores exceeding 32. Following MAF filtering, 20.8 million nuclear variants were included in statistical analyses. Among these, 44,460 variants (SNPs and indels) differed significantly between RER and control horses, including 385 predicted to have a high biological impact. Mitochondrial sequencing quality was high (Q > 32), with a mean depth of 280.9 ± 35.2X. A total of 434 mtDNA variants were identified at 410 positions, predominantly SNPs. Forty-two mtDNA variants differed significantly between groups, including three predicted to have high biological impact. Discussion & Conclusion: Whole-genome sequencing quality and mtDNA depth were high, ensuring complete coverage of the mitochondrial genome and consistent with expectations for blood-derived samples. Despite limitations related to sample size and annotation resources, this study provides valuable insights into candidate nuclear and mitochondrial variants associated with RER. Further analyses, including segregation studies, validation by Sanger sequencing and large-scale population genotyping will be required to confirm the best candidate variants. Given the exercise-induced nature of RER, mitochondrial DNA represents a biologically relevant target for investigation. These preliminary findings demonstrate the utility of whole-genome sequencing for characterizing mtDNA variability and identifying all putative genetic factors involved in RER susceptibility. References: Isgren CM et al. (2010) Norton EM et al. (2016) Barrey E et al.. (2011)

Prevalence of equine missing eyes (Microphthalmia and Anophthalmia) with sex skewed inheritance and parent-of-origin effect

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Eye diseases encompass a wide range of conditions, with two specifically related to abnormal eye development: anophthalmia, the complete absence of the eye, and microphthalmia, defined as an eye axial length 2 standard deviations below the population mean. These conditions have been reported in humans, with prevalence estimated to be between 0.002% and 0.017%, classifying them as rare diseases. While no conclusive genetic causes have been identified, several factors have been suggested to predispose individuals, including maternal age, Vitamin A deficiency, and infections during pregnancy. In horses, these conditions represent a significant portion of ocular diseases (7-14%), although specific prevalence rates have not been reported. The aims of this study were to estimate the prevalence of missing eyes (Microphthalmia and Anophthalmia) in Swedish Warmblood horses (SWB) and to investigate potential factors contributing to the disease. Information on cases has been recorded since 1997; however, systematic recording began in 2017. Thus, to obtain a realistic representation of prevalence, the study population comprised foals born between 2017 and 2023 (21014) along with their parents. Among those, 44 foals, sired by 28 different stallions, were affected. Analyses investigating the overrepresentation of sire lines among affected families, assessing inbreeding and relatedness, and evaluating the effect of age at mating were carried out in R (version 4.5.0). We estimated the prevalence of missing eyes in SWB to be 0.21%, with a higher proportion of affected females (95%) than males, suggesting that autosomal recessive inheritance or classical X-linked inheritance is unlikely and favouring epistatic interactions between at least two loci. The rates of unilateral and bilateral missing eyes were similar, accounting for 54% and 46%, respectively; all unilateral cases involved the left eye. This laterality might result from random developmental processes, potentially influenced by epigenetic regulation or X-inactivation. Among sires, the ones with at least one affected foal were significantly more related to each other (p-value = 1.47 x 10□□) than sires with no affected foals (IBD = 0.011). Obligate carrier sires were significantly overrepresented among the stallions (p = 7.25×10□□), although no single sire showed a large effect alone. Among female obligate carriers, older age at mating increased the risk of having an affected foal from 17% to 80%. A possible explanation for this pattern may involve age-related biological changes affecting female reproductive biology, such as oocyte quality, epigenetic DNA modifications, increased chromosomal variation, or reduced reproductive efficiency. Equine microphthalmia is a complex disease influenced by multiple factors. Based on our calculations, equine microphthalmia is no longer considered a rare disease. This shift is causing economic losses within the horse sector, and breeding associations should take action to control this disease.

Long-read mitochondrial DNA sequencing for genetic variability analysis in endurance horses

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Introduction: The mitochondrial oxidative potential plays a major role in skeletal muscle metabolism and is a key determinant of individual variation in endurance performance. Therefore, mitochondrial DNA (mtDNA) genetic polymorphisms warrant significant attention in studies of endurance in animals. However, research has largely focused on phylogenetics or haplotype diversity, and only a few investigations have analysed the contribution of mtDNA variants to performance traits. Moreover, studies of horse mtDNA are largely limited to small populations or partial genomic regions. To our knowledge, even fewer studies have combined a comprehensive intraspecific mitochondrial pangenome with GWAS for athletic traits. In a previous study of 430 endurance horses using short-read sequencing, mitochondrial GWAS identified 1,268 SNPs, 15 of which were significantly associated with endurance traits (raw p-value < 0.01), including average speed and finishing status. The large number of SNPs and alleles per locus highlighted the need to expand the study to a larger cohort to generate the first large-scale equine mitochondrial pangenome and explore mtDNA variants in greater detail. In addition, the horse genome contains a large nuclear insertion of mtDNA (NUMTS), which can confound variant calling accuracy. This motivated the use of a high-performance long-read sequencing methodology to identify mtDNA variants in more horses. Methods: We selected a new cohort of 300 horses to generate the first large-scale equine mitochondrial pangenome. We used long-read sequencing technologies combining nuclear DNA depletion and mtDNA enrichment via multiple displacement amplification (MDA). We optimized the multiplexing step to ensure full coverage, high depth, and cost-effectiveness. Results: We optimized and streamlined the molecular biology protocols to selectively amplify horse mtDNA and sequence it. From the first 108 samples, we achieved complete coverage of mtDNA in all cases, with a median depth of 457 and a median mapping quality of 59.5. We could call variants confidently across all mtDNA features, including the D-loop. Ongoing analyses include mitochondrial GWAS and comprehensive structural and sequence characterisation. Conclusion: Preliminary sequencing and analytical pipelines are complete, and detailed results will be presented at the congress, demonstrating both technical innovation and biological discovery. This integrated approach will identify mtDNA variants linked to endurance and provide novel insights into mitochondrial function, equine health, and athletic performance.

Systemic Transcriptomic Mirroring: Unveiling the Neuro-Muscular Axis and Non-Invasive Biomarkers for Elite Racing Performance in Horses

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Selecting elite Thoroughbreds currently relies on pedigree and phenotypic evaluation, but the underlying molecular architecture remains partially understood due to the invasiveness of skeletal muscle biopsies. This study aimed to identify systemic genomic signatures of racing excellence by integrating skeletal muscle and peripheral blood transcriptomes from 28 high-depth libraries. Using the Jeju horse (Natural Monument No. 347) as an unselected evolutionary baseline, we characterized the derived bioenergetic specialization in Thoroughbreds. Our analysis revealed a significant “transcriptomic mirroring” effect (R=0.63) between muscle and blood, identifying 89 core performance-associated signatures. Weighted Gene Co-expression Network Analysis (WGCNA) pinpointed a primary module (Darkorange2) driven by a synchronized neuro-muscular signaling axis, with key hubs including MAPK3, PHKA2, SBK3, and VAMP2. In silico source tracking (NNLS) mathematically confirmed that muscle-derived transcripts constitute a distinct reservoir (5–15%) within the circulatory system. Notably, blood-based levels of SBK3 and VAMP2 demonstrated near-perfect diagnostic accuracy (AUC = 0.97–1.00) for distinguishing elite runners. Furthermore, cross-breed meta-validation using an independent Arabian horse cohort confirmed PHKA2 as a universal bioenergetic engine ($p<0.001$), while identifying MAPK3 and VAMP2 as Thoroughbred-specific sprinting signatures. These findings validate peripheral blood as a high-fidelity window into intramuscular status, providing a welfare-friendly genomic framework for precision selection in the equine industry.

Circulating miRNAs and biochemical markers to evaluate heat-stress responses in Sicilian horse breeds

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The two Sicilian horse breeds, Sanfratellano (SAN) and Purosangue Orientale (PSO), represent valuable genetic resources adapted to harsh Mediterranean conditions. The analysis of circulating microRNA (miRNA) and biochemical parameters was conducted to establish functional links between gene regulation and physiological adaptation in heat-stress condition. A total of 15 PSO and 6 SAN were monitored during the thermoneutral TT and heat-stress HS seasons. Overall, 42 serum samples were collected and stored at -80°C until use for analysis on circulating miRNA and 84 serum samples, collected at the beginning and end of experimental tests per season, were used for clinical biochemistry. A Linear Mixed Model (LMM) for repeated measures was used to analyse biochemical parameters collected, including breed, season, and breed-season interaction as fixed effects and Temperature Humidity Index (THI) as a covariate. The subject was treated as a random effect to account for repeated measures and significance was assessed using False Discovery Rate (FDR) adjusted p-values. The QIAseq miRNA library kit was used for library preparation, followed by sequencing on the Illumina NovaSeq X Plus platform in 150-bp single-end mode. After extracting unique molecular identifiers (UMIs) and subsequent trimming, an average of 20.4 million high-quality reads were obtained per sample. About 1.18 million reads mapped to miRNAs. Additional small RNA classes were detected, including tRNAs (about 11.0 million reads), rRNAs (about 1.31 million reads), and other non-coding RNAs. Following DESeq2 normalization, miRNAs with a FDR lower than 0.05 and |log2 fold change| $\geq$ 1 were considered significantly differentially expressed. A significant effect of breed was observed for several biochemical parameters, with the SAN breed exhibiting higher values than PSO for Glutamate-Oxaloacetate Transaminase (GOT) and Gamma-Glutamyl Transferase (GGT), suggesting increased hepatic/oxidative activation and a more marked variation in some electrolytes and minerals (Mg,Ca). PSO generally showed a more stable and less pronounced profile for enzymes indicative of metabolic stress. Notably, GOT, GGT, alkaline phosphatase (ALP), total proteins and electrolytes counts were higher in the HS season than in the TT season, likely reflecting seasonal systemic response to heat load in both breeds. In the breed-season interaction SAN showed a more marked increase in liver enzyme values (GOT, GGT, LDH), Mg and Ca values during HS than in TT season, while in PSO a modest variation was observed indicating a minor biochemical response to heat-stress. Differential expression analyses across HS and TT seasons identified 23 (PSO) and 40 (SAN) differentially expressed miRNAs associated with the regulation of energy metabolism, inflammatory processes and immune response. PSO showed a more targeted modulation of immune-metabolic and stress adaptation pathways than SAN, which reported a more pronounced response. Furthermore, 5 miRNAs shared between breeds with opposite trends (upregulated in PSO and downregulated in SAN), indicating different post-transcriptional regulation of the mechanisms involved in the heat-stress response. This approach, integrating miRNA and biochemical markers, allowed the identification of season-associated genetic modulation patterns potentially linked to heat-stress adaptation mechanisms, showing greater physiological resilience in the HS season of PSO than SAN. This research was funded by “Progetto EquiDiv”-Istituto Incremento Ippico per la Sicilia and PRIN 2022-“ECOS”.

Selective breeding in insects - state of the art and prospects

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Advances in insect selective breeding and quantitative genetics are reshaping the potential for commercial production systems for insects used as food and feed. Recent reviews outline a selective breeding framework adapted to insects, including definition of breeding objectives, standardized phenotyping protocols, mating designs, and estimation of genetic parameters. This framework also emphasizes the necessity of mitigating inbreeding and maintaining genetic diversity in commercial insect populations. Insect species used for food and feed typically have short generation intervals. This provides benefits in terms of fast genetic progress, but it also complicates breeding as little time is available for phenotyping. Studies on insect species, such as the house fly (Musca domestica), the black soldier fly (Hermetia illucens), and the yellow mealworm (Tenebrio molitor), demonstrate the presence of additive genetic variance across relevant production traits such as survival, development time, and larval biomass. High-throughput phenotyping of many individuals simultaneously is critical for estimation of reliable heritabilities, variance components, and genetic correlations in these species, where environmental variance constitutes a large component of the phenotypic variation. Breeding programs must be designed specifically for each species, taking their species-specific reproduction and rearing requirements into consideration. Demonstrated genetic gains in insect populations confirm the feasibility and economic relevance of structured genetic improvement programs in insects. Yet they also reveal that there is room for improvement when it comes to selective breeding strategies and population management. Collectively, these developments establish selective breeding in insect production as an emergent discipline positioned to optimize future insect-based food and feed systems.

Global patterns of genomic variation in ten domesticated Black Soldier Fly (Hermetia illucens) strains

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The black soldier fly (BSF, Hermetia illucens) has positioned itself rapidly as a crucial species in sustainable biomass conversion, yet the genetic context underlying the performance differences of domesticated BSF strains remains poorly characterized. To broaden our current understanding of BSF population genomics, we analyzed genome-wide variation across ten laboratory-maintained domesticated populations originating from Europe (n = 4), Africa (n = 3), and Oceania (n = 3). Our aims were to characterize genetic diversity, quantify differentiation, and uncover major population structure patterns across these lineages. For each population, 20 individuals were sequenced using Illumina paired-end 150 bp whole-genome sequencing. Following stringent filtering and SNP discovery against the iHerIll2.2 BSF reference assembly, we quantified intra-population diversity, evaluated levels of genetic differentiation, and inferred evolutionary relationships. Population structure and relatedness were assessed through PCA, admixture modelling, and phylogenomic reconstructions. Additionally, scans for putative selection signals and runs of homozygosity were performed to compare these populations. The comparative analyses reveal substantial heterogeneity in genetic diversity and structure among globally sourced BSF strains, reflecting their distinct geographic origins and domestication trajectories. This work delivers one of the most comprehensive genomic assessments of BSF populations to date and provides a valuable foundation for future efforts in selective breeding, strain management, and optimization of bioconversion performance.

Estimating variance components and breeding values in honeybee populations via spatially aware simulation

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Simulations are an essential tool in animal breeding for time- and cost-efficient hypothesis testing. The key feature of breeding programme or population simulators is the temporal component of the simulation – we select the best individuals as the parents of the next generation to advance the improved genetic stock and increase genetic gain. In contrast, the spatial component is usually not considered or enabled by the simulators. Larger managed livestock is in general not able to freely move or mate, hence there might be no need to account for the animals' position in space beyond belonging to a certain management group, such as a herd. However, the spatial component becomes an important factor in the simulation of populations that can move around the space and whose environment cannot be completely controlled. The spatial component has two parts to it. The first one is the spatial genetic component, where relatedness depends on the distance between organisms, and the second is the environmental component, where the environment continuously changes through space. In honeybees, there are also many populations that collect phenotypes, but lack the pedigree information or at least the paternal side. This can be because part of the population is open-mated, but still recorded, or because there is not pedigree keeping. Such populations need to rely on genomic information for relatedness calculation. The aim of this work was thus to evaluate the choice of model for genomic evaluation of variance components and breeding values in an open-mated honeybee population via spatially-aware simulation. We simulated the population with the SIMplyBee R package. The simulation mirrored a yearly cycle of a managed, yet open-mated honeybee population. In the simulation, every colony was assigned a location and spatial mating was implemented by considering mating partners in a 5km radius. We simulated a single phenotype composed by the queen and worker trait. The phenotype was computed as the sum of queen and worker trait genetic effect, non-genetic systematic effect, spatial effect, and a residual. The spatial effect was simulated as a continuous spatial variation with R package INLA. The estimation models included the same components using an alternative Bayesian approach (Integrated Nested Laplace Approximation with Stochastic Partial Differential Equation, INLA-SPDE). The estimation scenarios varied the genotyping strategy by collecting i) only queen genotypes; ii) pooled genotypes of n workers per colony with varying n; and iii) queen and pooled workers genotypes. The models were evaluated by their performance in estimating variance components and breeding values. We used the continuous ranked probability score metric to evaluate the accuracy of estimated variance components by comparing the realised variance to the variance obtained by sampling from the posterior distribution. We estimated the accuracy of breeding values by correlating them with the true genetic value. Our results shed light into the sampling scheme required to reliably estimate quantitative genetic parameters in open-mated honeybee populations and will therefore guide future research as well as breeding efforts.

Integrative multi-omics analysis reveals genetic variants associated with growth traits in the superworm (Zophobas atratus)

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Abstract: The rising global population and increasing numbers of livestock are expanding the demand for efficient production of protein and other nutrients, creating a need for sustainable alternatives to cover this demand. Edible insects represent a promising future food and feed source due to their high nutritional value, efficient feed conversion, short generation intervals and potentially reducing the carbon footprint. Despite growing interest in insect-based production systems, genomic resources and functional genetic studies remain limited for many commercially relevant species, including the superworm (Zophobas atratus). This research study focuses on the darkling beetle species Z. atratus at the larval stage, aiming to characterize population-level genetic variation and to identify variants associated with growth-related traits, specifically body weight and length. To address this, we applied a multi-omics approach integrating genomics, transcriptomics and 3D epigenomics profiling to advance the functional interpretation of the desired loci and to identify key regulators of developmental pathways during larval growth. In a genome-wide-association-study (GWAS) based on whole-genome sequencing data, we identified SNPs linked to larval body weight and length. Functional prioritization is being achieved by integrating RNA-sequencing and high-throughput chromatin conformation capture (Hi-C) data to assess growth-dependent gene expression (eQTLs) and the spatial chromatin context of GWAS loci within topologically associating domains (TADs). This integrative framework allows the identification of regulatory variants whose genomic position and chromatin modification context suggests a functional impact on growth-related gene expression. In addition, diversity analysis reveals distinct kinship patterns and major genetic clusters within Z. atratus, providing insights into population structure and relatedness. Our work represents the first population-scale and GWAS-based investigation of growth traits in this species. Beyond advancing fundamental insect biology, these insights highlight the translational potential of omics-guided strategies for improving sustainable food and feed systems and addressing global protein demands. This work exemplifies how innovative omics technologies applied to non-traditional model organisms can contribute to translational research with relevance to human and animal health.

On the change of heritability and genetic correlations over time in honeybee breeding

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Heritability of a trait within a species is not a fixed biological constant but a population-specific parameter that depends on the genetic architecture and allele frequencies of a defined breeding population at a given time. Accurate estimation of heritability is essential for reliable breeding values estimation and for assessing expected genetic gain. Likewise, precise knowledge of genetic correlations between traits is crucial for the efficiency and predictability of simultaneous selection. As selection alters allele frequencies and genetic variance components, both heritabilities and genetic correlations are expected to change over time.Reliable estimation of genetic parameters requires breeding data of sufficient depth and structure. Broad datasets spanning multiple years increase statistical robustness and reduce sampling variance. Conversely, analyses restricted to shorter time intervals are more sensitive to temporal fluctuations in variance components. This creates an inherent trade-off: extended time horizons support stable parameter estimation, whereas shorter windows allow the detection of dynamic changes in genetic architecture.In this study, we analyse temporal patterns of heritabilities and genetic correlations for key performance and behavioural traits in the A. m. carnica breeding population, including honey yield, gentleness, calmness, swarming inertia, hygienic behaviour (pin test), and Varroa infestation development. The population is characterised by several decades of systematically recorded breeding data (Hoppe et al., 2020, Insects 11:768), enabling estimation of variance components across different temporal scopes and the assessment of their dependence on the selected time span and reference year.Across traits, we identify a consistent biphasic pattern in heritability development. An initial acceleration phase is characterised by increasing heritability, likely reflecting the reduction of environmental variance relative to additive genetic variance and the structuring of the breeding population under directional selection. This is followed by a saturation phase marked by declining heritability, consistent with a reduction in additive genetic variance as favourable alleles approach higher frequencies. Traits with lower initial heritability exhibit a delayed transition into the saturation phase.Genetic correlations show a complementary temporal pattern. In early phases of structured breeding, correlation estimates display comparatively large fluctuations, reflecting unstable covariance structures and sensitivity to sampling and selection effects. Over time, these correlations tend to stabilise and converge toward narrower value ranges, indicating consolidation of the underlying genetic architecture under sustained selection pressure.The results provide a population-genetic interpretation of long-term selection dynamics in the Carnica breeding population. Beyond the specific case studied, the analysis offers methodological guidance for breeding populations with shorter or less comprehensive datasets, particularly regarding the choice of temporal scope for parameter estimation and the interpretation of time-dependent genetic parameters.

Genotype × Environment and the gut microbiome shape Hermetia illucens performance: insights from 14 Lines across three diets

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The black soldier fly (Hermetia illucens) has become an important species for sustainable biomass conversion, yet its performance in rearing systems remains variable. Earlier studies have shown that both substrate and genetic background strongly influence larval growth, feed conversion, and waste reduction. These strain-specific differences are often accompanied by distinct gut microbiome profiles, some of which persist across generations, suggesting that certain microbes may be linked to host lineage. Such patterns point to a more intricate genotype × environment (G×E) interaction than previously assumed, with microbial communities potentially actings as intermediaries connecting substrate conversion, host genetics, and larval performance. To broaden our understanding of these interactions, we expanded our earlier work to eleven geographically diverse BSF populations and three controlled crosses, reared on three contrasting diets commonly used in BSF research and industry. Larval growth was measured daily, and pooled samples were subjected to PacBio long-read microbiome sequencing achieving high taxonomic and functional resolution. Early analyses reveal clear performance differences among strains on the various diets. Even within a single substrate, the microbiomes of different strains retain unique features. These early results suggest that specific bacterial taxa correlate with improved larval growth, hinting at beneficial microbial functions. By combining long-read microbiome sequencing with multi-strain phenotyping, this study aims to identify the microbial and genetic factors that make certain strain–substrate combinations more efficient than others. Ultimately, these insights may support the development of microbiome-aware breeding strategies for industrial BSF production and inform prebiotic treatments to improve substrate conversion. This highlights the importance of considering GxE interactions when selecting or developing strains for specific substrate-reducing applications.

Genetic progress in honeybee population under selection depending on mating design – a simulation study

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Breeding program of Apis mellifera is fundamental for enhancing traits related to productivity, docility, and disease resistance. A critical component of any breeding scheme is the degree of mating control, which determines both accuracy and genetic progress. This study presents a structured honey bee breeding program employing a dual mating strategy that integrates natural mating at a controlled mating station with a 70% reliability and instrumental insemination for precise pedigree management. Four scenarios were simulated: a baseline relying solely on the mating station (II0), and three scenarios in which 10% (II10), 20% (II20), or 30% (II30) of virgin queens were instrumentally inseminated each generation. Simulations assumed 1,000 colonies over 10 generations, each colony consisting of one queen and 100 worker bees. Queens were mated with 15 drones sourced from the top 10% of colonies. Each generation, 500 virgin queens were mated. The trait of interest had a mean of 0, genetic variance of 0.3, residual variance of 0.7, and no environmental effect. Trait architecture was modelled using 100 QTLs per chromosome (1,600 total QTLs). Results represent the average of 10 iterations per scenario, and genetic progress was evaluated relative to the baseline. Genetic progress increased steadily across all scenarios. As expected, both genetic gain and selection accuracy improved with higher proportions of instrumentally inseminated queens. By generation 10, scenario II30 achieved the largest improvement, reaching 50% greater genetic progress than the baseline, corresponding to 1.89 units. Scenarios II20 and II10 yielded 33% and 15% higher genetic progress than II0, respectively. Accuracy increased slightly until generation 3 and then stabilized, ranging from 0.27 in II0 to 0.44 in II30. In conclusion, the results suggest that combining a moderately reliable mating station with targeted instrumental insemination can optimize the efficiency of the honeybee breeding program. However, because instrumental insemination is costly, an economic evaluation should be performed before determining the optimal proportion of queens inseminated each generation.

Evidence for adaptation to divergent diets in black soldier flies using an experimental evolution set up

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The Black Soldier Fly (BSF, Hermetia illucens L.), can convert waste-stream products into valuable protein for food and feed. However, waste-stream-based diets pose challenges for the industry, as they often have low nutritional quality and can vary in composition over time. This may have negative effects on key production traits, including growth, development time, and fertility. It may be of interest for the industry to develop BSF-lines tailored to perform under low-quality diets. In this study, we aimed to adapt BSF-larvae to a high-quality diet and a low-quality diet using a low-cost-low-input experimental evolution setup. The idea being that long-term rearing on the two distinct types of diets would lead to local adaptation, i.e. better performance on their "home-base" diet. Six replicate lines were established from a commercial base population. The larvae of three lines were fed with a nutritionally rich diet with the main ingredient being organic alfalfa (ALF-1, ALF-2, ALF-3), and the other three lines with a nutritionally poor diet consisting of organic orange peels (ORA-1, ORA-2, ORA-3). In each generation, 1000-1500 flies were selected at random and mated to create the next generation. When the experiment concluded, the ALF-lines had been kept for 20 generations, and the ORA-lines for 15 generations. After the experiment, all six lines were subsequently reared on chicken feed for two generations. After those two generations, larvae from each line were exposed to two treatments: their own "home-base" diet or the alternative diet (e.g., ALF-1-alf or ALF-1-ora). From each line, for each of the two diet treatments, 50 larvae were selected at random and reared individually on either of the two medium types until adulthood. These larvae were weighed daily until they reached their top weight. When flies hatched, they were sexed, and a random selection of females was submitted to a heat tolerance test. Individual weight data for 563 larvae (249 females, 243 males, 71 unknown) were collected. Of the 563 larvae, 492 pupated and hatched as flies. Overall, female larvae had a higher top weight and reached their top weight later (μ = 158 mg in 12.5 days) than males (μ = 134 mg in 11.3 days). When fed alfalfa, ALF-larvae and ORA-larvae reached a similar top weight (μ = 146.9 gr and 146.2 mg, respectively), but ALF-larvae reached their top weight a bit earlier than ORA-larvae (μ = 7.6 days and 8.2 days, respectively). Larger differences were observed when larvae were fed with orange peels, where the ORA-larvae reached top weight faster but had a much lower top weight (μ = 127 mg in 15.2 days) compared to ALF-larvae (μ = 155 mg in 16.4 days). Finally, the ORA-larvae had a similar heat tolerance across diets and tended to have a higher heat tolerance than ALF-larvae. Yet, the heat tolerance of ALF-larvae tended to increase when they were fed with orange peels. In conclusion, these results show signs of evolutionary adaptation after relatively few generations of rearing on divergent diets. However, there is no support for the hypothesis that performance is higher when lines are tested on the diet they have evolved under. These findings provide promising insights for genetically selecting BSF through a low-cost-low-input selection process.

Determining the Minimal Viable Population Size of the Black Soldier Fly (Hermetia illucens) over Ten Generations of Inbreeding

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The production of black soldier fly larvae (BSFL, Hermetia illucens) is widely used for organic waste conversion and as a sustainable biomass source, particularly for animal feed. As production systems scale up, maintaining healthy and genetically stable colonies becomes crucial. However, it remains unclear how breeding population size affects long-term strain performance. To address this knowledge gap, breeding populations of 1000, 200, and 20 individuals were established in winter 2025. Each population is maintained for ten generations under standardized rearing conditions, with a 50:50 sex ratio and in triplicate, to systematically compare survival, performance, and genetic stability across population sizes. Prior to the start of the experiment, an optimized breeding protocol was developed for smaller populations. Initial trials showed that even when providing the calculated amount of feed per larva, survival was lower due to factors such as fungal growth. The protocol ensures that even the smallest populations can be reliably maintained while supporting consistent development and survival. This study aims to provide preliminary insights into the relationship between breeding population size, performance, and genetic stability in BSFL. In generations 1, 5, and 10, key traits including egg production, survival, growth, larval mass, pupal emergence and feed conversion efficiency are being monitored. By integrating phenotypic and genomic data, the project aims to determine a minimal viable population size and generate practical guidance for maintaining robust BSFL strains in both research and industrial production systems.

Circular Feed Ingredients in Livestock Systems: Opportunities and Constraints for Circular Agriculture

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Improving the circularity of livestock production systems is increasingly recognised as a priority for enhancing resource efficiency, reducing environmental impacts, and strengthening the resilience of agricultural supply chains. Feed production plays a central role in this challenge, as livestock diets rely heavily on a limited number of ingredients, including soybean meal and fishmeal, which are associated with land-use pressures, marine resource constraints, and supply volatility. At the same time, large volumes of nutrient-rich by-products and residues are generated across food processing, agriculture, and other industrial sectors. Circular feed ingredients offer a pathway to valorise these side streams by converting them into inputs for livestock and aquaculture nutrition. Traditional by-products such as brewers' grains and oilseed meals have long demonstrated the role of co-products in animal feed. More recently, technological innovation has expanded the range of residues that can potentially be redirected into feed supply chains. Emerging approaches include microbial fermentation to produce single-cell proteins and oils, enzymatic hydrolysis of animal and plant residues into functional feed ingredients, insect bioconversion of organic side streams, and thermochemical processes that convert biomass into feed additives. The aim of this presentation is to examine the potential role of circular feed ingredients in advancing circular livestock systems. Drawing on recent research conducted by The Centre for Feed Innovation and developments across the feed sector, the presentation will provide an overview of emerging technological pathways that enable the valorisation of underutilised biomass streams. It will discuss how these innovations may contribute to improving nutrient cycling, diversifying feed supply chains, and enhancing resource efficiency within livestock production systems across sectors including poultry, pigs, ruminants, and aquaculture. The presentation will also consider practical constraints influencing the adoption of circular feed ingredients, including regulatory frameworks governing feed safety, feedstock availability and logistics, technological maturity, and production economics. Understanding both the opportunities and limitations of these approaches is essential for identifying realistic pathways toward more circular and resilient livestock nutrition systems.

Food loss and waste to animal feed – an imperative for a circular, sustainable, One Health food system

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Food loss and waste (FLW) along entire supply chains from production to post-consumption accounts for 33 to 40% of total food produced for human consumption and is the greatest contributor to the inefficiency of resource use in global food production systems. About 25% of freshwater, 30% of agricultural land area, and 38% of total energy consumption used for food production is wasted due to FLW. About half of the greenhouse gas emissions from the entire global agrifood system are attributed to FLW, which represents 17% of total anthropogenic emissions. Progress toward achieving the Sustainable Development Goal 12.3 of halving global per capita food loss and waste by 2030 has been minimal. Therefore, increased efforts are needed to recover and recycle nutrients in FLW for other productive purposes. After reducing FLW and feeding hungry people, the next highest priority of FLW is to recycle nutrients in animal feed. Very few practices could be more impactful for reducing the environmental footprint in food animal production than repurposing FLW to animal feed in a circular bioeconomy. Recent results from modeling data obtained from more than 90 studies showed that recycling food waste into animal feed was carbon neutral and could result in average emissions mitigation of -1059 kg CO2equiv. per tonne of FLW by avoiding landfill disposal. If equal amounts of FLW currently disposed in landfills was diverted to aerobic composting, anaerobic digestion, and animal feed, GHG emissions in the EU would be reduced by 20 Mt CO2e which would offset 5% of total GHG emissions from agricultural systems. Under this same scenario, greater reductions in GHG emissions could also be achieved in the US (10%) and China (17%). By diverting one third of the FLW away from disposal in landfills, open-dumps, and incineration and using it in animal feed, about 9.4% of cropland, 9.7% of freshwater, 8.3% of energy, 6.3% of synthetic fertilizer, and 13.6% of herbicide and pesticide use attributed to maize and soybean production would be spared in the EU. Using greater amounts of FLW in animal feed would dramatically reduce reliance on imported feed ingredients, enable greater resiliency to feed supply chain disruptions, and substantially reduce the environmental burden of meat, milk, and eggs production. Unfortunately, only about 5% of the more than 58 million tonnes of FLW produced in the EU is used in animal feed, compared with Japan, which recycles about 55% of total FLW into safe, high quality, branded, and certified "Eco-feed". There are many reasons for the limited recycling of FLW to animal feed in most countries including: negative perceptions and historical images of "garbage feeding" to pigs; historical animal disease outbreaks and economic losses associated with feeding uncooked food waste to animals; competition with abundant, consistent supplies of commodity grains and agri-industrial by-products; lack of collection, handling, transport, and processing infrastructure; challenges of managing high moisture content, storage stability, and variability in nutrient content; lack of government and economic incentives; and overly restrictive laws and regulations. However, about 86 to 88% of total global FLW is derived from plants which have very low risk of microbiological feed safety concerns. Recycling FLW into animal feed and diverting potentially high feed safety risk animal-derived FLW sources to biogas production using anaerobic digestion, and soil amendments using aerobic composting can have an immediate impact and substantially improve the sustainability of food animal production.

Qualitative and quantitative analysis of meat by-products in France to enhance circular bioeconomy

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Animal by-products are materials of animal origin not intended for human consumption like (leather, wool, feathers, bones, blood, feet, heads, viscera). Materials posing a health risk are destroyed (e.g., coming from animals dead from disease…), while healthy materials are used in many industries (e.g., petfood, aquaculture, textile, organic fertilisers, biofuels, chemistry and medicine as for example heparin production…) and play an important role in a circular bioeconomy. The work carried out by GIS Avenir Élevages aimed to quantify and map the flows of these by-products, across all animal sectors: beef and dairy cattle, ovine, caprine, pork and poultry meat production. This analysis is based on the annual reports of the "Syndicat des Industries Françaises des Coproduits Animaux", supplemented by interviews with industry experts. By-products represent 55.0, 30.5 and 46.5% of annual slaughtered liveweight respectively for ruminants, pigs and poultry. In 2023 (https://terriflux.com/portfolios/IDELE/diagrams.html) the meat industry generated more than 2.0 million tonnes of by-products used as processed animal proteins, animal fats and bones, of which more than 800,000 tonnes came from ruminants. For the beef industry, the transition to negligible Bovine Spongiform Encephalopathy risk status has made it possible to classify more animal by-products as 'healthy'. Leather represented 161,000 tonnes and 441,000 tonnes were used through spreading and composting as fertilizers. Although synthetic substitutes exist for certain animal by-products, their performance and the quantities produced often make them the material of choice. The downward trend in livestock numbers and its predicted acceleration according to various forward-looking scenarios to reduce the carbon footprint of the livestock sector, could lead to supply problems for certain industrial sectors that depend wholly or partly on animal by-products. The socio-economic issues surrounding these products need to be addressed, alongside environmental issues, in order to assess their overall contribution to a circular bioeconomy.

Competitive resources in dairy cattle feeding: effect on yields, efficiency and emissions

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The use of resources (feed, land) competitive with direct human use has been increasingly questioned in terms of appropriateness and food-system level efficiency. Additionally, livestock systems are pursuing more environmentally friendly configurations. The aim was to assess the effect of the inclusion of competitive feed resources in lactating dairy cow's diets on milk energy yield (MEY), net gross energy (GE) provision, and greenhouse gas emissions (GHGe). Data were collected from 336 farms, representative of northern Italy systems. Farm visits were performed to obtain data on farm size, milk yield and quality, animal diets as well as management and structure to compile farm inventory. Daily MEY was computed as MY x GE content (NASEM protocol). Lactating cows' dry matter intake (DMI) was computed based on ratio between net energy requirement and dietary net energy. The GE intake (GEI) was computed as DMI x dietary GE content, considering the whole diet (GEIw), contribution due to concentrates target of land occupation (GEIc) and to human-edible portion of the diet (GEIe). Net provisions were calculated as the difference between MEY and GEIw (NEPw), GEIc (NEPc) or GEIe (NPEe). Life Cycle Assessment was applied to compute GHGe (as 100-y global warming potential, GWP) per kg of fat- and protein-corrected milk (FPCM), considering cradle-to-farm gate boundaries, biophysical milk-meat allocation and IPCC computation protocol. Classes of percentage of GEI covered by GEIc (GEIc_p) and GEIe (GEIe_p) (quintiles, average values of 4(6), 17(19), 24(25), 31(30), and 42(39)%, for quintiles from 1 to 5 of GEIc (GEIe)) were used in two separate mixed models to test their effect on MEY, NEPs and GWP. Quartile of farm size, lactation stage and percentage of specialized breeds as well as use of total mixed rations (yes vs no), housing (tie vs loose stall) and random effect of the dairy system were used as additional effects. Average size was 101 lactating cows (min-max: 3-1009), MY 25.7 kg/cow/d (8.5–44.5) and MEY 78.2 MJ/cow/d (25.3–135.5). Net provision (mean±sd) averaged -345±38 (NEPw), -23±48 (NEPc), -21±37 (NPEe). Mean GWP was 1.48±0.28 kg CO2-eq/kg FPCM. The GEIc_p significantly affected MYE, NPEs and GWP. In particular, MYE and GWP in first quintile were significantly lower (MYE) and higher (GWP) than 3rd-5th quintiles, with intermediate values in 2nd quintile. About NEPs, increasing GEIc_p was associated with a linear increase in NPEw and decrease in NPEc, with values above zero in the first two quintiles (i.e. positive net provision of energy). Same results were found for GEIe_p. In conclusion, diets with GEIc_p close to 17% and GEIe_p to 19% resulted in the best combination of high MYE, low GWP and positive energy net provision when feed origins and types were considered. Moreover, although not completely overlapping, concentrates could be used as proxy of human-edible portion of the diets, allowing faster and easier evaluation of the lactating cows' diets. Acknowledgement: project 'Looking for the agro-environmental friendly dairy farm: innovative approaches and integration of methodologies for an inclusive definition of sustainability in dairy systems', Prot. 2022ME2537, call PRIN 2022, fundend by European Union – NextGenerationEU.

Closing the loop in Mediterranean lamb production: Circular economy benefits of cucumber–straw silage evaluated through in vitro, in vivo, and life cycle assessment

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Improving efficiency while reducing environmental impacts remains a major challenge for livestock systems in Spain, where viable alternatives to conventional forages are limited. This study evaluated cucumber–straw silage (SIL) as an alternative forage to oat hay (HAY) for lamb production using an integrated approach combining in vitro rumen fermentation, an in vivo commercial farm trial, and a cradle-to-farm-gate life cycle assessment (LCA). In the in vitro batch culture experiment, concentrate was progressively replaced with SIL or HAY at 25, 50, 75, and 100%, and ruminal fermentation characteristics, cumulative gas production, and methane (CH_4) emissions were measured over 144 h. Subsequently, a 12-week in vivo trial was conducted on a commercial farm with 450 lactating Segureña ewes and 610 lambs to assess productive performance and metabolic status. The in vitro experiment followed a factorial design (two forage types × four inclusion levels) within a completely randomized design, while the in vivo experiment was performed using a completely randomized design. Results showed that a 50% inclusion of SIL maintained fermentative activity comparable to HAY, including total gas production and total volatile fatty acid concentrations; however, SIL shifted fermentation toward greater butyrate production (+13.3%; P = 0.001) and reduced CH_4 emissions (−17.3%; interaction P = 0.001), indicating a potentially more favourable rumen fermentation profile. In vivo, SIL supported comparable animal performance to HAY, with no significant differences in lamb ADG (210 vs. 215 g/day; P = 0.382) or ewe body condition (P = 0.954), and blood metabolites remained within physiological ranges, indicating adequate metabolic health. A cradle-to-farm-gate life cycle assessment, following ISO-14044 standards and applying two allocation approaches for cucumber waste, revealed that under a zero-allocation scenario (treating waste as burden-free), SIL reduced greenhouse gas emissions by 18% (9.50 vs. 11.6 kg CO_2-eq per kg liveweight), land occupation by 50%, and water consumption by 70% compared with the HAY-based system. Therefore, SIL potentially presents an effective strategy to valorise horticultural waste while maintaining productivity and improving the environmental performance of Mediterranean lamb systems. Further validation across diverse management conditions and by-product sources is recommended.

Advantages of an ensiled total mixed ration including sugar beet pulp for French dairy farms

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In the current context, environmental and economic concerns encourage the development of sustainable solutions in dairy farms. Among these solutions, the inclusion of byproducts in total mixed rations (TMR) has gained increasing attention in many countries. In addition, ensiling TMR can reduce labour requirements and simplify feeding practices. Between 2017 and 2024, the benefits of ensiled TMR with sugar beet pulp versus fresh TMR were assessed in France through experimental trials and field surveys, focusing on cow performance and feeding management. First, a preliminary study was conducted with two groups of cows (35 cows per group; 1 Normande, 5 Jersey, 29 Holstein) comparing an ensiled TMR (Pulp'Mix®, Tereos) and a fresh TMR over 6 months. The diet was based on grass silage, maize silage, sugar beet pulp, and distillers dried grains with solubles (DDGS). Cows fed the ensiled TMR showed a numerical improvement in feed efficiency, without any decrease in milk yield. Daily milk fat production tended to increase ($P = 0.09$). These results were reinforced in a study conducted in 2020, with two groups of cows (11 Holstein cows per group) fed an ensiled TMR or a fresh TMR, with the same ingredients (mainly maize silage, sugar beet pulp, baled herbage, DDGS, wheat gluten feed), for 53 days. When expressed per kg of dry matter intake, milk yield and milk fat were higher in cows fed the ensiled TMR ($P < 0.05$) compared to the control group. In addition, methane emissions, measured using a GreenFeed system, were significantly reduced, both per day ($P < 0.05$) and per kg of milk ($P < 0.01$), in the group fed the ensiled TMR. Increase in the efficiency of cows and reduction in methane emissions may be attributed to a shift of some fermentation processes from the rumen to the silo. In particular, the higher lactic acid concentration in the ensiled TMR could enhance the propionic acid production and reduce the methanogenesis. Regarding field surveys, before/after comparisons (i.e. before and after the introduction of the ensiled TMR with sugar beet pulp and various byproducts) were conducted in 2017 in Alsace-Lorraine, in 2018 in Hauts-de-France, and in 2024 in Hauts-de-France. In 2017, 9 dairy farms were evaluated, allowing statistical comparisons. Results showed a significant reduction in feeding costs ($P < 0.05$), as a result of a higher inclusion of wet byproducts. Using an ensiled TMR instead of mixing all ingredients every day resulted in time savings estimated at more than 90 h per year. Similarly, in the 2018 survey, performed in three farms, time savings were evaluated between 90 and 120 h per year. In addition, milk yield was maintained, with an increase in feed efficiency. Feeding costs were reduced. Finally, the 2024 survey conducted in three farms estimated time savings of around 80 h per year. Before/after comparison in one farm showed stable milk and stable fat productions over the year. In conclusion, studies and surveys over the past years demonstrated the advantages of an ensiled total mixed ration based on byproducts, such as sugar beet pulp, in dairy farms. This solution can not only reduce labour requirements but also lower feed costs, and represents a sustainable option through a circular local economy and decreased methane emissions.

Ensiling by-products: destoned olive pomace silage as an on-farm strategy for lamb meat production to reduce food/feed competition and enhance meat quality

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Tuscany is among the leading Italian regions for olive oil production and sheep farming, two activities that are historically rooted and remain central to the region's agricultural identity and economic value. These sectors generate large amounts of olive pomace (OP) as a by-product (BP) and contribute to a high demand for animal feed. Although OP is recognized as a sustainable feeding resource for ruminants the perishability and seasonal availability limit its widespread use. Drying is commonly adopted to stabilize feed, but for water-rich BP this approach can negatively affect feasibility and sustainability due to high energy requirements. Mixing different BPs and ensiling them represents an on-farm solution to stabilize water-rich BP with low economic and environmental costs. This study evaluated the productive performance and meat quality traits of Appenninica lambs fed diets based on destoned OP silage, wheat bran, and beet pulp combined with distillers' grains (SD) or faba beans (SF), compared with a conventional diet (C) based on maize, barley, soybean, and alfalfa hay. A completely randomized design was applied to 18 lambs (average body weight: 18.8±2.1 kg), equally allotted to three dietary treatments (SD, SF, and C). After the adaptation period, data were collected over four weeks. Dry matter intake (DMI) and water intake (WI) were daily recorded while body weight (BW) was weekly recorded. The offered feed and refusals were analysed for chemical composition to assess nutrients intake. After slaughter, carcass weights were recorded, and meat samples were collected for laboratory analyses. A linear mixed model was applied with diet and week as fixed factors, animal as random factor, and initial BW as a covariate. Model for analysis of meat quality traits included diet as a fixed factor and animal as a random factor. DMI (934±30 g/d) did not differ among treatments. The C diet showed lower aNDF intake ($291\pm12_{b}$) respect to SF ($354\pm12_{a}$) and SD ($332\pm12_{ab}$). Similarly, ADL intake was lower in C ($14\pm1_{b}$) than in SF and SD ($36\pm1_{a}$). Ether extract intake was higher in SF and SD ($64\pm1_{a}$) than in C ($26\pm1_{b}$). Crude protein intake was lowest in C ($103\pm4_{c}$), intermediate in SF ($125\pm4_{b}$), and highest in SD ($164\pm4_{a}$).WI (L/kg DMI) was significantly greater in SF ($4.2\pm0.4_{a}$) than in C ($2.8\pm0.4_{b}$), whereas SD were intermediate ($3.6\pm0.4_{ab}$). No differences were found in final BW (24.6±0.6 kg), average daily gain (201±19 g/d), carcass weight (12.2±0.6 kg) among treatments. Chemical composition of meat did not significantly differ among treatments, except for total lipid content that was higher in SF ($4.1\pm0.4_{a}$) respect to SD ($2.3\pm0.4_{b}$) and C ($3.6 \pm 0.4_{ab}$). No significant differences were observed for meat colour parameters and deoxymyoglobin but oxymyoglobin was highest in C ($78.4\pm2.5_{a}$), followed by SD ($73.5\pm2.7_{ab}$) and SF ($67.0\pm2.7_{b}$). Metmyoglobin was higher in SF ($15.2\pm2.8_{b}$), followed by SD ($9.8\pm2.8_{ab}$) and C ($3.4\pm2.5_{a}$). Unsaturated fatty acids (FA) ($43.9 \pm 1.3_{a}$) and monounsaturated FA ($37.6 \pm 1.5_{a}$) were significantly higher in SF than in C and SD, whereas polyunsaturated FA did not differ among treatments. The ω-6/ω-3 ratio was lowest in SF lambs ($4.6 \pm 0.6_{b}$) compared with C ($6.9 \pm 0.6_{a}$) and SD ($6.9 \pm 0.7_{ab}$). Ensiled OP in combination with other BP can be successfully included in the diet of fattening lambs without any detrimental effect on growth performance if compared to a conventional diet. Overall, using ensiled OP in the lamb diet enhances nutritional quality of meat minimizing food–feed competition.

What intrinsic levers do mixed crop–livestock farmers in Guadeloupe have to improve the agroecological performance of their farms?

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Meeting the growing demand of Guadeloupean consumers for healthy, affordable, locally produced food is constrained by limited and shrinking agricultural land, part of which is durably contaminated. This calls for efficient farming systems dedicated to supplying the local market, which currently has a self-sufficiency rate of only about 25%. Family farming represents 80% of farms in the archipelago and produces a wide diversity of products in small quantities, mainly marketed through short supply chains (local markets, direct sales). Crop–livestock integration (CLI) has the potential to enhance the agroecological performance (efficiency, resilience, autonomy) of small tropical family farms. We evaluated the multi-performance of small organic farms in Karukera (Guadeloupe) according to their degree of CLI and diversification (MAKIBIO). With the support of the GDA ECOBIO network of organic farmers, participatory surveys were conducted with 29 farmers across the archipelago between 2024 and 2025. Farms were selected to represent the diversity of local CLI practices. Farm performance was assessed using the STREAM tool developed within the European Agroecology-TRANSECT project and adapted to tropical conditions. Agroecological indicators were derived from ecological network analysis (ENA) and expressed as annual nitrogen flows. The field phase was conducted using a participatory approach: half a day was devoted to a task chosen by the farmer before carrying out questionnaires and observations. The involvement of 5 to 6 researchers over an extended time fostered trust, common language and improved understanding of farm organization. The surveyed farms averaged 9 ha of area (over 40% ≤6 ha) and employed 1.98 workers on average. The Shannon index of cultivated plant biodiversity ranged from 0.002 to 2.57 (mean 1.24), while the Shannon index of livestock biodiversity (corrected for tropical livestock units) ranged from 0 to 1.44. Internal nitrogen circularity (internal flows/total flows) ranged from 8% to 83%, reflecting a wide range of CLI practices. However, no direct relationship was found between ENA-based agroecological performance and biodiversity indices or nitrogen circularity. The type of livestock enterprise had a stronger influence on nitrogen-use efficiency than the degree of crop–livestock integration. Intensive organic monogastric systems rely on high inputs but achieve high productivity, whereas grazing ruminant systems require fewer inputs and generate greater within-system circularity. Farm resilience increased strongly with the balance of flows between farm compartments ($R^2 = 70.11$; $p < 0.001$). To a lesser extent, resilience, efficiency and autonomy increased with the number of farm compartments and their interconnections. These results highlight that resilience largely depends on the farmer's systemic vision of the farm. In this tropical context, the independence between resilience and nitrogen-use efficiency appears to result from recycling processes associated with increasing agroecosystem complexity. In the medium term, the collective aims to digitalize the STREAM tool to monitor small farms over time and support farmer-led data collection for decision-making.

Energy-Plus Dairy Farming – Evaluating the Decarbonization Potential of Integrated Biogas and Photovoltaic Systems in Northern German Milk Production

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The transition towards a low-carbon agriculture requires dairy farms to evolve from pure food producers into integrated energy hubs. While livestock farming is traditionally associated with high greenhouse gas (GHG) emissions, the strategic implementation of large-scale renewable energy systems – specifically biogas and photovoltaics (PV) – offers a transformative pathway to offset these impacts through the circular economy and avoided burdens. This study investigates the environmental performance of nine intensive dairy farms in Northern Germany, focusing on the mitigation potential of high-capacity renewable energy integration. The research quantifies how on-farm energy generation affects the global warming potential (GWP), acidification (AP), and eutrophication (EP) of 1 kg of milk. Individual farm data from the calendar year 2024 or the financial year 2023/24 were used. Cradle-to-farm gate life cycle assessments (LCA) were conducted respectively. The functional unit was defined as 1 kg energy-corrected milk. Economic allocation of 86:12:2 (milk, culled cows, calves produced) was applied. Four technical scenarios were compared: (0) integrated PV and biogas (1) biogas only, (2) PV only, and (3) a baseline reference without renewable energy systems. The integrated energy system (Scenario 0) achieved a 27% reduction of GWP compared to the baseline. The biogas plant was identified as the primary mitigation driver, accounting for 44% of total GWP savings. This is attributed to the "double-dividend" of biogas: the prevention of methane (CH_4) leakages from manure storage and the subsequent generation of carbon-neutral electricity. While PV systems provided a significant further reduction (approximately 15% of total savings), their impact was strictly limited to electricity substitution. Changes in AP and EP were marginal (1–2%), primarily driven by the reduction of fossil-fuel combustion in the national grid. The study demonstrates that large-scale energy integration can pivot Northern German dairy farms into "Energy-Plus" operations. The findings highlight that the synergy between manure-based biogas and solar energy is essential for reaching decarbonization targets in the livestock sector. This research provides a robust framework for policymakers and farmers to evaluate the dual role of agriculture in food security and renewable energy transitions.

Effects of nitrogen fertilization, biorefinery processing and season on nitrogen fractions in grasses

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This study aimed to evaluate how grass species, nitrogen fertilization level, cut number, biorefinery processing, and alkaline treatment during biorefinery affect total N concentration (total N) and the proportion of neutral detergent insoluble nitrogen (NDIN) relative to total N (NDIN-P) in grasses and pulp/press-cake. Treatments included three grasses (perennial ryegrass and festulolium [festuca and lolium type]), three levels of fertilization (300, 450, and 600 kg N ha^{-1} $year^{-1}$), and four fractions (original plant, pulp pressed once [pulp 1], pulp pressed twice with water [pulp control], and pulp pressed twice with NaOH [pulp alkaline]). All treatment combinations were evaluated across five cuts in 2024 (May 14, June 11, July 9, August 12, and September 23). The complete random design resulted in 180 samples (3 species × 3 N fertilization levels × 4 fractions × 5 cuts). Following the first pressing, pulp was mixed with either deionized water (Milli-Q) or a 0.6% NaOH solution, kept overnight at room temperature and pressed again (pulp control and pulp alkaline, respectively). Fractions were freeze-dried for analysis of neutral detergent fiber (NDF, Fibertec) and total N (Dumas). Nitrogen was determined in the NDF residues to calculate NDIN-P. Statistical analyses were performed in R using linear mixed-effects models (lmer) including Species, N fertilization level, Cut, Processing, and their two-way interactions as fixed effects. Species x N fertilization level x Cut was included as a random effect in the model. Pairwise comparisons were performed using Tukey-adjusted tests. Interactions were observed for total N between N fertilization × Processing ($P<0.01$) and Cut × Processing ($P<0.01$), and for NDIN-P between Species × N fertilization ($P<0.01$), Species × Processing ($P<0.01$), N fertilization × Processing ($P<0.01$), and Cut × Processing ($P=0.01$). Within processing, interactions with level of fertilization were detected only in the original plant, where total N increased with N fertilization rate (from 1.88% of DM at 300 kg N ha^{-1} to 2.81% of DM at 600 kg N ha^{-1}). NDIN-P was higher at 300 and 450 kg N ha^{-1} (≈45% of N) compared to 600 kg N ha^{-1} (35.8% of N), with no differences observed among processed pulps. In general, total N concentration decreased with increasing processing intensity in all cuts and N fertilization rates, with pulp 1 and pulp control consistently showing intermediate and similar values. The tendency for NDIN-P was the opposite, increasing with increasing processing intensity (74.4% of N on pulp alkaline vs. 42.0% of N on original plant). Increasing fertilization correlated positively with total N, mainly by increasing the soluble fraction. The biorefinery process efficiently extracts N, and the alkaline treatment further removes N. Festulolium festuca type showed a higher NDIN-P (64.3% of N) than Ryegrass and Festulolium lolium type at 600 kg N ha^{-1} only. Within Processing, NDIN-P was higher in Ryegrass in the original plant (49.1% of N) and in Festulolium fescue type in pulp 1 (62.6% of N). Interactions with Cut were observed only in the original plant material, where total N was higher in cuts 2 and 3 (≈2.8% of DM) than in cuts 1, 4, and 5 (≈2.0% of DM). Cut 2 also had a lower NDIN-P (30.3% of N) compared to cuts 1, 3, 4, and 5 (≈45% of DM). The lower total N content in Cut 1 relative to Cut 2 and 3 was unexpected and might be due to lower fertilization level or later-than-intended harvest, potentially reducing soluble components. In conclusion, cut and N fertilization primarily affected the soluble N fraction. As the season progressed, total N decreased, due to a decreased content of soluble N.

Greenhouse gas emissions of grass-based dairy systems: insights from nutrient cycle assessments of Dutch dairy farms

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Grasslands contribute to many ecosystem services and play a crucial role in achieving (inter)national sustainability goals for e.g. biodiversity, water quality and carbon sequestration. Besides that, these grasslands offer non-human edible feed resources which ruminants can convert into high quality human edible food. However, the utilization of grass by ruminants contributes to emissions of greenhouse gasses (GHG), nitrate and ammonia, which have negative environmental impacts. This study, therefore, addresses how milk (and meat) production from Dutch grasslands can be optimized for low GHG emissions while simultaneously considering other sustainability indicators, including ammonia emissions, soil nitrogen surplus and protein self-sufficiency. We selected 18 conventional dairy farms (9 on clay and 9 on sandy soil) with a high, medium, or low proportion of total grass (fresh grass, grass silage and hay) in the cow's diet. We quantified technical and environmental performances of 2023 and 2024 of these farms using the Annual Nutrient Cycle Assessment (ANCA) tool. In ANCA, GHG emissions are quantified via a life cycle assessment approach. At these farms, total grass in the diets varied from 39 to 78% on clay and from 28 to 62% on sand. Diets with a low proportion of grass contained more maize silage and byproducts, while the proportion of concentrates was relatively similar between the different diets (20-30%). With increasing percentages of grass in the diet, annual milk production per cow decreased from about 11,000 to 8,100 kg fat protein corrected milk (FPCM) on clay and from 10,400 to 9,500 kg FPCM on sand. Total GHG emissions per kg FPCM varied and increased slightly with more grass in the diet (4.3g CO2-eq kg FCPM^{-1} per percentage point increase in dietary grass proportion, hereafter %$^{-1}$), while GHG emissions per hectare decreased with more grass in the diet (-0.25t CO2-eq ha^{-1} %$^{-1}$). On average, 46% of the GHG emissions originated from rumen fermentation, increasing slightly with higher percentages of grass in the diet (2.1g CO2-eq kg FCPM^{-1} %$^{-1}$). 25% of the GHG emissions originated from external inputs, on average decreasing with more grass in the diet (-1.1g CO2-eq kg FCPM^{-1} %$^{-1}$). The rest of the GHG emissions originated from on farm feed production, increasing slightly with more grass in the diet (3.9g CO2-eq kg FCPM^{-1} %$^{-1}$), from energy use and from the barn and manure storage, both decreasing slightly with more grass in the diet (-0.3g CO2-eq kg FCPM^{-1} %$^{-1}$ and -0.3g CO2-eq kg FCPM^{-1} %$^{-1}$, respectively). These results indicate that increasing the dietary grass proportion shifts the relative contribution of GHG emission sources, suggesting trade-offs between enteric, on-farm and external emission components. High proportions of grass in the diet were associated with higher protein self-sufficiency (0,69% %$^{-1}$), while ammonia emissions decreased slightly (-0,54 kg NH3 ha^{-1} %$^{-1}$) and no consistent relationship was observed with soil nitrogen surplus. The observed patterns indicate that higher dietary proportions of grass can be associated with improved performances of several sustainability indicators at farm level. Furthermore, the observed variation between farms indicates that there is room to optimize sustainability indicators across a range of dietary grass proportions. This suggests that that a relatively high proportion of grass in dairy cow diets does not necessarily result in higher GHG emissions per kg FPCM, while more grass-based systems can contribute to more ecosystem services and substantially reduce feed–food competition of milk and meat production.

It's not always as simple as it seems: lessons from genetic susceptibility to prion and lentivirus infections in sheep

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Classical scrapie and small ruminant lentivirus (SRLV) infections in sheep are among the few infectious diseases in which a single genetic variant can play a decisive role in determining host susceptibility. For classical scrapie, selection for the amino acid arginine (R) at codon 171 of the prion protein (PrP) was a milestone in the control and eradication of this disease from many sheep populations. Likewise, the prevalence of SRLV infection was shown to be significantly lower in sheep homozygous for lysine (K) at codon 35 of the transmembrane protein 154 (TMEM154). Despite these clear associations, breeding for lower susceptibility to both diseases is not always straightforward. A major limitation is the low frequency or absence of the protective allele in the target population. In Iceland, unsuccessful efforts have been underway for almost 50 years to eradicate scrapie in sheep. However, a survey in 1999 dashed hopes for a breeding solution, because no sheep carrying the R171 allele were identified in the Icelandic sheep population. In search of an alternative, we conducted a case-control study including 4,782 samples, of which 360 originated from scrapie-positive sheep, from 20 scrapie-affected Icelandic flocks. The results suggest that alternative PrP variants at codons 137, 138, 151 and 154 are associated with lower susceptibility than the wild type. The threonine (T) variant at codon 137 proved particularly promising, as it was not detected in any scrapie-positive sheep. Moreover, it behaved similarly to R171, acting as a strong molecular barrier to prion conversion in the protein misfolding cyclic amplification (PMCA) assay. After genotyping almost 30,000 Icelandic sheep, only a few R171 carriers were found. Therefore, T137, C151 and H154 are now considered protective alleles in Iceland. For SRLV susceptibility, the protective TMEM154 K35 allele also occurs at low frequency, particularly in susceptible breeds. In addition, a frameshift mutation in exon 1 showed a protective effect in German Pomeranian Coarsewool sheep. However, in some breeds, such as German Merinoland sheep, no significant association of TMEM154 E35K with SRLV susceptibility was observed despite sufficiently high allele frequencies. Similar to ovine scrapie, where susceptibility to atypical forms is not governed by the same PrP variants as in classical scrapie, the protective effect of TMEM154 variants may depend on the genotype of the circulating SRLV. Recently, SRLV genotype B2 was detected together with A5 in a Merinoland flock, and both genotypes infected sheep with TMEM154 genotype KK. Thus, viral genetic variation alone does not explain the missing protective effect. In summary, these examples emphasize the importance of considering additional genetic variation in both the host and the pathogen.

Dissecting the genetic architecture of somatic cell score in Cyprus Chios ewes: Heritability, genetic correlations with milk traits and GWAS analysis results using 50K SNP DNA microarray data

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Somatic cell score (SCS) is an important indicator of udder health and subclinical mastitis in dairy sheep, and high SCS significantly impacts milk quality. Data for about 900 Cyprus Chios ewes with parities 1 to 6 were analyzed in this study. Genetic analyses showed moderate heritability for SCS ($h^2 = 0.31 \pm 0.03$) and high repeatability ($r = 0.66 \pm 0.008$). Genetic correlation analyses revealed that SCS was strongly and negatively associated with lactose content at both the genetic and phenotypic levels ($rg = -0.49 \pm 0.07$, $p = 3.95 \times 10^{-12}$; $rp = -0.37 \pm 0.02$, $p = 7.21 \times 10^{-74)}$. Total milk yield also exhibited a negative association with SCS at the genetic level ($rg = -0.29 \pm 0.08$, $p = 4.48 \times 10^{-4}$) and at the phenotypic level ($rp = -0.16 \pm 0.02$, $p = 3.40 \times 10^{-12}$), reflecting a strong antagonism between udder health and milk production. Furthermore, this study investigated further the genetic architecture of SCS in Cyprus Chios sheep using a combination of linear and binomial GWAS models and 50K SNP microarray genotypes for 721 Cyprus Chios ewes. While binomial scans under strict Bonferroni correction identified high-confidence hits on chromosome 10 within the Basic Immunoglobulin-Like Variable Motif Containing (BIVM) gene and a Chromosome 6 hotspot involving non-coding RNAs, linear models were refined using a multi-marker backward elimination procedure (Multi-Locus Mixed Models). This approach identified 11 independent SNPs explaining 55.42% of the total genetic variance for SCS. Functional annotation of these regions revealed a large set of genes associated with milk quality and mammary health. Significant findings include the gene CCSER1, encoding a protein that is a crucial regulator of milk content and udder shape, and CEP112, previously linked to mammary morphology traits. The NRG3 gene, which encodes a cell-cell signaling protein and has been connected to mammary gland conformation and reproductive performance, was also identified in our analysis. In addition, an important mediator of the NF-kB inflammatory pathway was identified. These results demonstrate how a multi-marker refinement approach effectively captures complex genetic signals and link for the first time genetic pathways involved in structural mammary characteristics, milk composition, and immune-related signaling to mastitis resistance in commercial dairy sheep, such as the Cyprus Chios breed.

Genetic selection as a sustainable strategy against gastrointestinal nematodes in Flemish Texel sheep

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Gastrointestinal nematodes, particularly Haemonchus contortus, pose an increasing threat to sheep production, compromising animal welfare and causing substantial economic losses. Across Europe, the cost of gastrointestinal nematode infections is estimated to €443 million annually in sheep and goats, with approximately 80% attributable to production losses and 20% to treatment expenses. The situation is further exacerbated by the emergence of anthelmintic resistance and by additional stressors such as the 2024 Bluetongue virus outbreak. In this study, 2,300 lambs, primarily from Texel flocks, were phenotyped between 2017 and 2025 in Flanders (Belgium). Owners participated voluntarily in the study, and the sampling aimed to include farms with pedigree links. Recorded traits included body weight, FAMACHA® score (anaemia indicator), body condition score (muscle and fat reserves), DAG score (extent of faecal soiling) and log-transformed faecal egg count (log(FEC)). The pedigree used in the genetic analyses included information tracing back five generations, including a total of 7,855 animals. A multi-trait animal model was fitted to estimate genetic parameters and breeding values using the BLUPF90 software. To account for non-heritable sources of variation, the model included breed, dam's age (6 classes), litter size at birth, sex, farm effect (defined as breeder + recording date) and lamb age fitted as both linear and quadratic covariates. Average standardized weight at sampling was 34.44 ± 7.65 kg at 140.65 ± 30.80 days of age. Heritability (h_2) estimates differed markedly among traits. Body weight ($h^2 = 0.40 \pm 0.05$) and DAG score ($h^2 = 0.19 \pm 0.04$) were moderately heritable, whereas FAMACHA ($h^2 = 0.05 \pm 0.02$) and log(FEC) ($h^2 = 0.07 \pm 0.02$) had low heritability. Moreover, favourable genetic correlations (rg) were observed: genetically heavier lambs tended to show lower parasite burdens (rg=-0.26 ± 0.15) and reduced anaemia indicators (rg=-0.15 ± 0.31). These findings demonstrate that progress in parasite resistance is associated with improved growth performance. A clear litter-size effect on body weight remained visible at sampling age, with lambs born as singles averaging +3.67 ± 0.31 kg, and those from triplet litters averaging -2.21 ± 0.39 kg, relative to twins. Predicted genetic gain expressed relative to the average age and weight of the lambs in the dataset, indicates that selecting the top 15% of animals could increase lamb weight by 2.67 kg while simultaneously reducing FEC by 12% within a single generation. The EBVs are particularly valuable for lowly heritable traits such as FEC and FAMACHA, where individual phenotypes provide limited information. Several improvements of the scheme are envisaged: genomic data has the potential to enhance selection accuracy, especially in young animals lacking own performance records. Secondly, a cost-effective and scalable phenotyping method would allow for sampling more animals and lead to stronger genetic gain. Finally, knowledge of the individual nemabiome and the parasite communities will refine the breeding values across farms. Overall, this study in a limited number of flocks demonstrates the potential of reducing parasite burden using selection and as such provides a realistic pathway towards more resilient, productive and sustainable sheep systems in Flanders.

Genetic correlations between dairy and maternal traits in the Lacaune dairy sheep breed

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In ruminants, there is a growing interest in producing meat from dairy herds. In France, lamb production from dairy ewes account for 23% of the national lamb production and crosses with meat sheep breeds are common to produce lambs with improved meat traits. Improving reproductive (i.e. prolificacy) and lamb meat traits in dairy herds might be relevant. However, the genetic potential of French dairy sheep breeds to produce lambs has never been evaluated. In this study we analysed data from more than 11,000 lambings and lactations and 13,000 lambs in the Lacaune dairy sheep breed, collected at the INRAE La Fage experimental farm. The objective was to estimate the heritability of prolificacy and early growth traits and their genetic correlations with the main selected dairy traits (milk yield, fat and protein yields). Prolificacy was modelled as dairy traits, with a direct genetic effect and a permanent environmental effect of the ewe, while early growth traits (measured on lambs) were modelled with direct and maternal genetic effects and a permanent environmental effect of the dam. The heritabilities for dairy traits, close to expected values, are moderate to high (25%, 56% and 65% for milk yield, fat yield and protein yield, respectively). The heritability of prolificacy reaches 9%, and birth weight is heritable both at the direct and maternal genetic components levels (27% and 20%, respectively). The direct heritability of body weight at 30 days of age (BW30) is higher than the heritability of the maternal genetic component (26% and 8%, respectively). For both studied body weights, negative genetic correlations were estimated between direct and maternal components (-0.28 and -0.12 for birth weight and BW30, respectively). Prolificacy is not genetically correlated with dairy traits (genetic correlations below 0.06). Milk yield is moderately positively genetically correlated with birth weight and BW30 direct and maternal components (genetic correlations ranging from 0.22 to 0.44). Genetic correlations between milk yield and maternal components of birth weight and BW30 decrease as days in milk increase (from 0.34 to 0.11 at 42 and 167 days in milk, respectively for birth weight; from 0.62 to 0.24 at 42 and 167 days in milk, respectively for BW30). Fat yield is only significantly genetically correlated with the direct genetic effect of BW30 (-0.19). Protein yield is moderately negatively correlated with direct genetic effects of both lamb traits (-0.36 and -0.19 for birth weight and weaning weight, respectively) and is moderately positively correlated with the maternal genetic effect of birth weight (+0.19). Traits related to lamb production are thus heritable in the Lacaune dairy sheep breed and are mostly positively correlated with dairy traits, which is favourable for their improvement in dairy sheep breeding programmes.

Genetic correlations between leather pinhole defect and selected traits in Lacaune dairy sheep

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Pinhole defect consists of small holes on the grain surface of a dewooled pelt, which negatively impacts the quality of lamb leather. This defect is increasingly found on Lacaune lamb skins, which are usually recognized for their suitability for processing into high quality leather, intended for the luxury industry. The occurrence of the defect often leads to the downgrading of the skins, affecting their market value. However, the valorization of livestock by-products is considered as a way of improving the sustainability of current sheep breeding systems. A first study involving around 1,400 Lacaune lambs, genotyped using the Illumina OvineSNP50 BeadChip and phenotyped for pinhole revealed a high heritability for this defect (0.59 ± 0.07), and a high genetic correlation with a wool trait, the predicted core bulk, that could be measured on live animals. To assess the impact of a selection on pinhole defect, genetic correlations between pinhole and traits currently included in the breed's total merit index were estimated. Phenotypes measured during the routine genetic evaluation for milk traits (milk yield, fat content, protein content, fat yield and protein yield), udder morphological traits (teat angle, anteroposterior teat position , udder depth and udder cleft), and lactation somatic cells score were used for around 8,000 ewes, which were full or half-siblings of the lambs phenotyped for pinhole. Genetic correlations between pinhole and milk quantity and quality traits appeared to be quite low and in a favorable sense, meaning that reducing the prevalence of the defect would not compromise milk traits. However genetic correlations with udder traits were higher and in an unfavorable direction, a better mammary conformation being associated with an increase of the defect. Further insights should be obtained by measuring leather phenotypes on more lambs coming from dams of the open nucleus flocks. More generally, our results may open up new prospects in other dairy sheep breeds for improving wool and leather quality, in order to obtain a better valorization of these farming by-products.

Genomic insights into health resilience and environmental adaptation in Portuguese and Angolan small ruminants

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Local small ruminant populations represent valuable reservoirs of genetic diversity shaped by natural and artificial selection under diverse production systems. Understanding the genomic basis of resilience to disease and adaptation to harsh environments is essential for sustainable breeding and conservation strategies. In this study, we integrated genome-wide analyses from European and African small ruminant populations to investigate genetic mechanisms associated with health and environmental adaptation. First, a genome-wide association study (GWAS) was conducted in Portuguese Merino sheep naturally exposed to coccidial infection to identify loci associated with parasite resilience. Using high-density SNP genotypes (~531K markers), several significant genomic regions were detected, including variants located within or near genes encoding for Claudin and Rab proteins, highlighting biological pathways related to epithelial barrier integrity, vesicle trafficking and immune signalling involved in host–parasite interactions. Second, high-coverage whole-genome sequencing (≈30×) was performed in 49 sheep and 42 goats sampled across multiple provinces and ecological regions of Angola, representing populations adapted to extensive pastoral systems and challenging climatic conditions. Preliminary selection scan analyses identified candidate genes under selection, related to growth hormone regulation, maternal processes involved in parturition and neuromuscular junction development, suggesting genomic adaptation associated with growth, reproductive performance, and structural resilience in these populations. Together, these results demonstrate how genomic analyses across contrasting environments can uncover complementary mechanisms underlying health resilience and environmental adaptation in small ruminants. The integration of GWAS and high-coverage whole-genome sequencing provides valuable resources to support breeding strategies and the conservation of locally adapted livestock populations.

From zero to hero – the spread of a new breed throughout a sheep industry using the Texel in Britain as an example

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Britain has traditionally had an extensive sheep industry spread throughout the farming sectors from mountain down to mixed lowland systems. Innovative farmers have always looked to improve profitability and sustainability of their enterprises by introducing new ideas and methods. In the case of the British sheep industry, one such innovation was the introduction of foreign breeds – in this particular case the Texel from The Netherlands and France. There were no known flocks of Texels in Britain until their introduction in early 1970s as a maternal breed playing a part in the stratified crossbreeding structure endemic in Britain. Regular surveys of the breeding structure of the British sheep industry over the last 50 years have highlighted the growth of the Texel breed in the country. The latest survey carried out in 2020 showed just how far the breed had spread throughout the various sectors of the British sheep industry. Although originally thought of as a maternal breed, on initial importation, the breed soon demonstrated a higher carcase lean content in subsequent carcase evaluations. This resulted in its widespread adoption as a sire of lambs for meat production. Thus by 2020 about 27% of the ewes in Britain were mated to Texel rams and their genes accounted for the largest breed contribution to lamb carcase meat. Whereas the Texel found its place on British farms as a terminal sire it was also discovered that the breed had suitable maternal qualities, the main reason for its original importation. By 2020 the breed contributed to over 2 million crossbred ewes mostly used as the mothers of lambs for meat production. Thus, from a starting point of zero in 1970 the breed has spread throughout the British sheep industry to contribute to both the paternal and maternal side of lamb production. Not surprisingly this has led to a reduction in the use of local breeds in these roles over this period (1970 to 2020). On the paternal (Terminal Sire) side the Suffolk breed has gone from over 50% of the national ram flock in 1970 down to about 9% in 2020. Whereas the Suffolk did not play such an important role in the maternal component of British sheep, nevertheless its role on the maternal side has declined too. Interestingly, the various breed surveys carried out over the last 50 years have highlighted over 26 imported breeds in Britain and another 16 synthetic produced locally; none of these other breeds or synthetics have achieved the same level penetration of the British sheep industry as the Texel. This dramatic uptake of a new breed over 50 years is a reflection of its qualities both as a contributor to higher lean proportion carcases and more efficient mothering abilities of the ewes. Clearly decisions in the early days of importation were based on objective measurement of the characteristics of a new breed and extensive highlighting of the advantages of using such a breed in the appropriate sectors of the industry.

Genomic insights into the genetic diversity of the Slovak Askanian merino sheep

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The Merino sheep is a globally recognized fine-wool population that has given rise to numerous Merino and Merino-derived breeds. The Askanian Merino is one such breed, established between 1925 and 1934 at Askania Nova in Ukraine and historically maintained in Eastern and Central Europe. However, in recent decades, changes in the wool industry and an overall decrease in the number of sheep in Slovakia have led to a dramatic decline in its population size. Currently, the Slovak Askanian Merino population is confined to only a few hundred animals. Therefore, this study aimed to characterize the genomic diversity of the Slovak Askanian Merino population using SNP data. After standard quality control, the dataset comprised 48,419 autosomal SNPs and 506 individuals, representing the entire active population of the Slovak Askanian Merino. Genetic diversity was assessed using the inbreeding coefficient based on homozygous-by-descent segments (FHBD) and the effective population size (Ne) estimated based on the relationship between linkage disequilibrium patterns and Ne. The Slovak Askanian Merino showed a total FHBD of 2.5%, while the most pronounced length category of the HBD segments contributing to overall inbreeding exceeded 16 Mb. These long HBD segments reflect recent common ancestry within the population. A difference in inbreeding was observed between sexes, with FHBD reaching 2.5% in ewes and 5.3% in rams. The higher FHBD observed in rams likely reflects the limited number of breeding males in the current population. This increases the probability of mating among related individuals and contributes to higher realized inbreeding in rams. The contemporary Ne was estimated at 78 individuals, while historical Ne estimates showed a decreasing trend over recent generations. The observed trends in Ne and FHBD are consistent with demographic changes in the analysed population and indicate a recent reduction in genetic diversity. These results suggest that targeted genetic diversity management is necessary to prevent further erosion of this unique animal genetic resource. This research was supported by the Ministry of Education, Research, Development and Youth of the Slovak Republic (grant no. 1/0316/25) and the Slovak Research and Development Agency (grant no. APVV-20-0161).

Genomic diversity and population structure of the endangered Thuringian goat breed

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The Thuringian goat is an endangered dairy breed from Germany that was developed in the 20th century through crossbreeding between local goats in Thuringia and the Swiss Toggenburg goats. It is valued for its high milk yield, fertility, hardiness, and adaptability to cold, mountainous, and harsh environments. This study aimed to assess the genetic diversity of the Thuringian goat and examine its genetic relationships with other European breeds, especially its historical ancestor, the Toggenburg goat, as well as to identify genomic regions under positive selection. Genetic analyses were conducted using SNP chip data from 1,233 animals representing 36 European breeds across 43 populations. After quality control, 47,040 SNPs were retained for downstream analyses. Genetic diversity was assessed using observed and expected heterozygosity and nucleotide diversity, while inbreeding was estimated from excess homozygosity. Relationships between Thuringian goats and other European breeds were analysed using FST, phylogenetic analysis, and PCA, and selection signatures were identified from RoH islands. The analyzed Thuringian goat population exhibited the lowest genetic diversity among all breeds, characterized by the lowest heterozygosity (36.61%), the lowest nucleotide diversity (6.37×10^{-6}), and a high inbreeding level (FHom = 18.2%). The Thuringian goat showed moderate genetic differentiation from the investigated European breeds, with FST values ranging from 0.104 with Alpine (Italy) and Saanen (France) to 0.126 with Malaguena (Spain), while Toggenburg populations showed FST values of 0.128 and 0.133 for the Swiss and Tanzanian populations, respectively. Principal component analysis (PCA) revealed that Thuringian goats clustered closely with Alpine (Italy), Saanen (France), and Toggenburg (Switzerland), whereas the Toggenburg population from Tanzania exhibited greater divergence. RoH analysis showed higher homozygosity in short regions (<1 Mb; FRoH = 4.33%) and lower homozygosity in longer regions (>4 Mb; FRoH = 3.22%). This pattern indicates mainly historical inbreeding in the Thuringian goat, whereas recent conservation efforts may have mitigated recent inbreeding. Genes located within RoH islands in Thuringian goats were mainly associated with mastitis and mammary innate immunity, lipid metabolism and oxidative homeostasis, immune and cellular regulation, fertility and reproduction, stress response, development, bone growth, and neurodevelopment. The low genetic diversity and high inbreeding observed in the Thuringian goat underline the need for targeted breeding and conservation efforts. Expanding the genetic base, strengthening breeder networks, and limiting further inbreeding will be crucial to safeguard the long-term viability and genetic integrity of this endangered breed.

From pedigree to genomics, studying genetic diversity and selection signatures in the Cornigliese sheep breed

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Sheep are among the most widespread species in the Mediterranean region and play a central role in traditional farming systems. According to the FAO, over 60% of Italian sheep breeds are classified as having an unknown risk status, and about 28% are considered endangered. Among these endangered breeds, the Cornigliese sheep is a local breed facing a population decline. The small population size and limited pedigree information make it challenging to control inbreeding and manage genetic diversity in this breed. Therefore, implementing effective conservation strategies is essential for ensuring the breed's long-term survival. The present study aims to investigate genetic diversity and selection patterns in the Cornigliese sheep breed using an integrated approach based on pedigree and genomic data. We used pedigree information from 5,784 animals and genomic data from 293 individuals genotyped with the Illumina® OvineSNP50v2 BeadChip. An additional 42 individuals from the Bergamasca breed were genotyped with the same SNP chip. All analyses related to pedigree completeness and genetic variability were performed using ENDOG (v4.8), while genotype data were analyzed in PLINK (v1.9) and R (v4.5.0). Pedigree-based inbreeding in Cornigliese sheep is notably low (2%). However, due to low pedigree completeness (CGE = 1.03), the reliability of these values is limited. In contrast, genomic inbreeding showed a higher level of inbreeding (10%), consistent with values reported for other Italian local breeds. Meanwhile, Bergamasca sheep exhibited lower genomic inbreeding (6%), consistent with their much larger population size. Our analysis also supports the historical and geographical connections between the two breeds examined. Population structure analyses revealed a close clustering of Cornigliese and Bergamasca sheep, reflecting their historical exchanges. ROH islands, shared by at least 70% of individuals within specific farms, were identified as candidate SNPs under directional selection. Selection-signature analysis identified several ROH islands, primarily on chromosomes 2, 6, and 9, where genes and quantitative trait loci (QTLs) associated with growth, carcass, milk, and wool traits are located. On chromosome 2, the TFPI gene was identified in the flanking region of an ROH island. TFPI is a plausible candidate target for selection related to growth and productive performance, as it has previously been associated with body weight in meat-oriented breeds across several species, including horses and cattle. The ROH island detected on chromosome 6 overlaps with a genomic region previously linked to wool quality in other sheep breeds, such as Mérinos, which historically contributed to the development of the Cornigliese breed through crossbreeding. In addition, within the ROH island on chromosome 9, the UBXN2B gene emerges as a promising candidate due to its reported association with carcass weight and intramuscular fat deposition. Overall, these findings reveal genomic regions influenced by both historical and recent selection, offering important insights to inform conservation programs and future genomic selection strategies for this endangered breed.

Predicting Feed Efficiency from Multi-Omics Data in Lambs Fed Two Consecutive Diets

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In sheep farming, improving feed efficiency can lower the carbon footprint, limit the feed-food competition and strengthen farmers' economic sustainability. However, few breeding companies can record individual feed intake, which is essential to estimate feed efficiency. Previous studies have identified feed efficiency proxies, such as plasma metabolites or rumen microorganisms. Nonetheless, it remains unclear whether proxies are robust across environments. Here, the goal was to assess whether feed efficiency proxies are generalizable across different years, diets and growing periods. Since 2015, Romane meat lambs have been divergently selected for residual feed intake at the INRAE P3R unit (https://doi.org/10.15454/1.5483259352597417E12). Between 2018 and 2022, 290 male lambs were raised under two different diets. First, lambs were accustomed to a 100% concentrate diet and were phenotyped in spring (17 to 23 weeks of age). Later, the same animals were fed a mixed diet (66% hay, 33% concentrate) and were phenotyped either in summer (29 to 35 weeks of age) or in fall (37 to 43 weeks of age). Under each diet, the collected phenotypes included body weight at the beginning and end of the 6-week period, as well as back ultrasound to measure muscle depth and backfat thickness. Daily intakes of concentrate and forage were recorded to compute the average daily energy intake (ADEIC under a concentrate diet, ADEIM under a mixed diet). From the previous phenotypes, residual energy intake (REIC or REIM) was calculated separately for each diet. Lambs were genotyped with a 54K SNP chip. After each 6-week period, the rumen microbiota was sequenced (16S V4-V5 regions), and the plasma metabolome was obtained using NMR spectroscopy. Partial least-squares models were used to assess the predictive ability of pedigree, genotypes, microbiota and metabolomic data separately. The prediction accuracies of REI and ADEI were computed by randomly subsampling the datasets 100 times and averaging the Pearson correlation (r) between the predicted and true phenotypes. Models were developed for each diet and also across diets, using proxies measured under one diet to predict phenotypes under the other. Whatever the diet, the best proxies for REI were the pedigree and the genotypes ($r = 0.56$ for REIC and 0.32 for REIM), followed by plasma NMR spectra ($r = 0.42$ and 0.26), and rumen microbiota ($r = 0.16$ and 0.19). When samples were collected under one diet, no reliable proxies could be identified to predict REI under the other diet. Whatever the diet, ADEI was better predicted by fixed effects and body weight ($r = 0.88$ for ADEIC and 0.78 for ADEIM). ADEI could be moderately predicted by omics data ($0.58 \leq r \leq 0.69$ for ADEIC, against $0.47 \leq r \leq 0.65$ for ADEIM). When ADEI was measured under one diet, proxies collected under the other diet could still moderately predict it. For example, ADEIM was partially predicted by the rumen microbiota composition under the concentrate diet ($r = 0.64$). Similarly, ADEIC was moderately predicted by the rumen microbiota under the mixed diet ($r = 0.69$). However, models selected different proxies depending on the diet. Finally, the prediction distributions suggest that microbiota data and NMR spectra mostly capture the environmental variations of energy intake, such as the year of rearing. Our results confirm that feed efficiency and energy intake proxies are context-specific: proxies could not be generalized across diets or environments. Thus, integrating new omics data into breeding programs would require collecting omics data routinely: each year and under each farming system.

Estimation of genetic parameters for meat traits using replacement ewe lambs.

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In the Lacaune meat sheep breeding program, run by the breeding society Ovi-Test, elite artificial insemination rams are evaluated based on the maternal abilities of their daughters in nucleus flocks. Concurrently, these elite rams are also evaluated for meat traits based on the phenotypes of their progeny in dairy ewe flocks. Ongoing developments within the Lacaune meat sheep breeding program, particularly the prospective transition from traditional to genomic selection, offer the opportunity to reorganize meat trait evaluation, potentially restricting phenotyping to nucleus flocks only. Ovi-Test has implemented a protocol for recording meat trait data on replacement ewe lambs. Since 2024, 1,421 ewe lambs from 16 flocks have been phenotyped on-farm for meat traits at approximately 110 days of age (mean = 108 days). Measurements included ultrasound scanning for fat (mean = 4.6 mm) and muscle depth (mean = 23.3 mm), muscle development scoring (five elementary scores rated from 1 to 10), and post weaning growth (mean = 168 g/day). Uni-trait genetic evaluations revealed heritability estimates close to those used in official evaluations. Estimated heritabilities for growth rate, fat depth, and muscle depth were 0.278, 0.274, and 0.375, respectively. Multi-trait models confirmed these estimates. Preliminary estimates of genetic correlations suggest an unfavourable genetic correlation between fat and muscle depth, however, the accuracy of these estimates is low and additional data are required to achieve sufficient precision. These initial results indicate that selection for meat traits, assessed through phenotyping of replacement ewe lambs, is feasible. Reproductive performance data from these ewe lambs as well as the pre-weaning growth of their progeny will enable a first estimation of genetic correlations between meat and maternal traits in the Lacaune meat breed.

Enhancing the sustainability of Latvian sheep breeding through genomic selection for relative growth rate and Kleiber ratio

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The function of breeders of sheep breeds is not only to produce economically valuable lamb meat, but also to improve the breed, especially in the case of national breeds. One indicator of breed improvement, which also contributes to breeders' income, is feed conversion relative to live weight gain, or breeding lambs with high feed efficiency. Marker-assisted selection (MAS) related to feed utilisation has been developed for commercial breeds, but it is not yet applicable to national breeds. The aim of the study was to identify molecular markers from the GeneSeek® Genomic Profiler™ Ovine 50K (Lansing, MI, USA), that are related to feed efficiency indicators: relative growth rate (RGR) and Kleiber ratio (KR) in Latvian sheep breeds, especially in the national breed. DNA genotyping data from 261 controlled fattening lambs of eight common breeds, including 135 (51.72%) of the national breed Latvian Dark-headed (LT), were used. The computed average RGR and KR for the 60-day period (from day 90 to 150) for all lambs were 0.40±0.12 % per day and 18.84±2.52, respectively; whereas for the LT breed, they were 0.36±0.08 % per day and 19.80±2.27, respectively. In the genome-wide association study (GWAS) analysis for association with RGR, 1876 SNPs were identified with $P < 0.01$ and 3709 SNPs with $P < 0.05$ across all breeds and 504 SNPs were identified with $p<0.01$ and 2014 SNPs with $p<0.05$ for the LT breed only. For association with KR, 2277 SNPs were identified with $P < 0.01$ and 3976 SNPs with $P < 0.05$ across all breeds and 526 SNPs were identified with $P < 0.01$ and 2053 SNPs with $P < 0.05$ for the LT breed only. GWAS results showed 4130 common loci for RGR and KR across all breeds, and 1472 loci for the LT breed only. Machine learning methods were employed to integrate lamb birth rates and GWAS-significant loci to identify the most effective predictors of the lamb RGR or KR prior to the fattening phase. The resulting MAS prediction would facilitate the identification of potential breeding candidates before they mature. The study revealed the potential of MAS to enhance national breeding efforts in Latvia, especially for the LT breed, thereby improving breed development and competitiveness while maintaining national value. Acknowledgements: Funded by UL Faculty of Medicine and Life Sciences internal student grant FUD2025/195, and by the Latvian Council of Science, Latvia, projects LZP-2021/1-0489 and LZP-2024/1-0092.

Genetic determinism of leather pinhole defect in Lacaune dairy sheep

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Lacaune lamb skins are renowned for their exceptional suitability for high-quality leather production, catering to the luxury industry. However, over the past few decades in France, the quality of this leather has declined significantly, primarily due to the increasing prevalence of a skin defect known as pinhole. This defect manifests as small holes in the grain surface of the dewooled pelt. To unravel the genetic determinism of this defect, 1,400 Lacaune lambs were genotyped using the Illumina OvineSNP50 BeadChip and phenotyped for the pinhole defect. A genome wide association study revealed a region located on OAR3 that influences this trait. Haplotypes surrounding the most significant SNP were reconstructed and evaluated using analysis of variance, leading to the identification of haplotypes associated with a less pronounced skin defect. Through the whole genome sequencing of 14 animals and the analysis of polymorphisms located within a 5 Mb region surrounding the most significant SNP, three candidate causal mutations were identified in keratin genes KRT1, KRT8 and KRT74. These mutations are non-conservative resulting in changes in the protein-coding sequences. To investigate the potential effects of these candidate mutations, controlled matings were conducted on an experimental farm to produce lambs homozygous for either the wild-type or the mutated alleles at the three loci. Skin biopsies were collected at 2 months of age from these lambs. After slaughter at the end of the fattening period, skins were tanned and visually assessed by experienced tanners for the presence of pinhole. Histological examination and gene expression analyses of the biopsy samples are currently being conducted to elucidate the molecular mechanisms underlying the presence of skin defects. This study brings to light the first evidence of genetic markers that can be used for the sorting of lambs with high potential for skin quality.

Effect of Aloe vera Supplementation in a Tris-Based Cryoextender on the Post-Thaw Quality of Box-Frozen Goat Spermatozoa

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Goat semen cryopreservation is an important reproductive biotechnological tool for sperm conservation. However, freezing–thawing can impair sperm function and structure. Cold shock, osmotic imbalance, and oxidative stress are major limitations for the successful cryoprervation for frozen semen in goats. Therefore, improving the composition of semen extenders is of considerable practical interest. Aloe vera, known for its antioxidant and protective bioactive components, may help reduce cryoinjuries, but there is limited evidence regarding its use in goat semen cryopreservation. This study aimed to evaluate the effect of Aloe vera supplementation in a Tris-based extender on the post-thaw quality of box-frozen goat spermatozoa. Semen samples were collected from three sexually mature Saanen bucks using an electroejaculator. Fresh semen was assessed before cryopreservation to establish baseline values. Post-thaw sperm kinetics were evaluated using the iSperm portable Computer-Assisted Sperm Analysis (CASA) system (Aidmics Biotechnology). Total motility and progressive motility were numerically lower in the Aloe vera group than in the control group (47.50 ± 4.79% vs 60.50 ± 2.40% and 43.25 ± 4.04% vs 56.25 ± 2.43%, respectively). Similarly, the number of live spermatozoa was lower in the Aloe vera group than in the control group (44.50 ± 3.30 vs 56.75 ± 3.20), whereas the number of dead spermatozoa was higher (72.75 ± 3.64 vs 58.75 ± 2.72). However, paired statistical comparisons showed that these differences were not statistically significant ($P > 0.05$). In conclusion, Aloe vera supplementation to the tested Tris-based extender did not improve the post-thaw quality of box-frozen goat spermatozoa under the present experimental conditions.

Genetic background of feed efficiency residual traits for sustainable breeding of sheep breeds raised in Latvia.

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Sheep breeders' responsibilities extend beyond just producing economically viable lamb meat. They also involve improving breed quality, especially for indigenous populations. One of the key indicators of breed improvement that can also increase breeders' income is feed efficiency relative to live weight gain, particularly through selecting lambs with higher feed efficiency. Marker-assisted selection (MAS) related to feed utilisation has already been developed for commercial breeds; however, it has not yet been adapted for national breeds in Latvia. The aim of this study was to identify molecular markers associated with residual traits of feed efficiency, specifically residual feed intake (RFI), residual weight gain (RWG), and residual intake and body weight gain (RIG) in sheep breeds that are raised and selected in Latvia, with a particular emphasis on the national breed, the Latvian Dark-headed (LT). Genotyping data collected using the GeneSeek® Genomic Profiler™ Ovine 50K (Lansing, MI, USA) were analysed from 261 performance-tested fattening lambs from eight common breeds, including 135 animals (51.72%) from the national Latvian Dark-headed breed. Across all lambs, the RFI and RWG values calculated for the 60-day period (from day 90 to 150) ranged from -0.16 to 0.14 (SD 0.04) kg per day and from -0.45 to 0.52 (SD 0.16) kg per day, respectively. For the LT breed, these values ranged from -0.11 to 0.11 (SD 0.04) kg per day. The ranges of RIG were from -5.51 to 3.44 (SD 1.65) across all lambs and from -4.50 to 3.46 (SD 1.62) in the LT group. The genome-wide association study (GWAS) results for RFI found 1,095 SNPs with a significance level of less than 0.01 and 3,068 SNPs with a significance level of less than 0.05 across all breeds; in the LT breed alone, there were 559 SNPs ($P < 0.01$) and 1,986 SNPs ($P < 0.05$). The analysis identified 2,524 SNPs with $P < 0.01$ and 4,058 SNPs with $P <0.05$ for RWG across all breeds; the LT population specifically exhibited 533 SNPs ($P < 0.01$) and 2,189 SNPs ($P < 0.05$). GWAS analyses found 1,118 loci linked to both RFI and RWG across all breeds, and 335 loci in the LT breed. For RIG, 1,539 SNPs ($P < 0.01$) and 3,305 SNPs ($P < 0.05$) were identified across all breeds; specifically, within the LT breed, 533 SNPs ($P < 0.01$) and 1,999 SNPs ($P < 0.05$) were identified. Of all the SNPs associated with RIG, 1,094 loci were also associated with RFI and RWG in the entire sample group, and 331 SNPs were found within the LT breed. Machine learning methods were used to combine lamb birth data with important genetic markers to identify the best indicators of RFI, RWG, or RIG before the fattening phase. The resulting MAS-based predictions would enable the identification of promising breeding candidates before they reach maturity. Overall, the study demonstrates the potential of MAS to strengthen national breeding programmes in Latvia, particularly for the Latvian Dark-headed breed by supporting genetic improvement, increasing competitiveness, and preserving national genetic resources. Acknowledgements: Funded by the UL Faculty of Medicine and Life Sciences internal student grant FUD2025/195 and by the Latvian Council of Science, Latvia, projects LZP-2021/1-0489 and LZP-2024/1-0092.

Mapping Growth and Carcass Traits in Sheep for Sustainable, Low Emission Meat Production.

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Sheep body weights, especially pre-weaning (pre-WW, 8 weeks), post-weaning (post-WW, 20 weeks) and carcass weight (CW), are key indicators of early development, feed efficiency, and market readiness. Improving these traits enhances productivity while also reducing emissions per kilogram of meat, as animals that grow fast require less feed and emit less. This study aimed to identify single nucleotide polymorphisms (SNPs) associated with body weight and carcass traits by utilizing single-step genome wide association study (ss-GWAS) and regional heritability mapping (RHM) methods. A high-quality dataset of 1,348 sheep with pre- and post-WW and 289 with CW, alongside 43,823 SNPs, was analysed. Both methods employed a mixed linear model with litter size, age at pre- and post-weaning, and age at slaughter as fixed effects. Borderline suggestive signals ($P < 2.3\times10^{-5}$) emerged for pre-WW including two SNPs on chromosome 3 and one SNP each on chromosome 4 and 20. One SNP on chromosome 17 surpassed suggestive significance for CW ($P < 2.2\times10^{-5}$). RHM analysis yielded multiple suggestive regions on chromosome 3 for pre-WW ($P < 0.005$), and chromosome 17 for CW ($P < 0.0002$). No significant SNPs were identified for post-WW from either method. Gene annotation within ±50 kb region of the SNPs identified 34 genes for pre-WW with five (DNAI7, IRAG2, ENSOARG00020084804, ENSOARG00020092445, ENSOARG00020065109) overlapped between ssGWAS and RHM. For CW, both ss GWAS and RHM highlighted MAML3 as an overlapping gene. Functional annotation of the overlapped genes from both methods indicated pre-WW genes were linked to ciliary movement and sensory perception, whereas CW genes were associated with gluconeogenesis, hepatic lipogenesis, and intramuscular fatty acid deposition. This study has demonstrated that the integration of ss-GWAS with RHM reveals single SNP and regional effects for sheep body weight and carcass traits, identifying genes that can support breeding of animals with greater efficiency, lower emissions, and improved carcass quality.

Genome-wide signatures of selection and genomic divergence in three autochthonous sheep breeds from Epirus, Greece

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Indigenous sheep breeds represent valuable genetic resources shaped by long-term adaptation to local environments and by human-driven selection for production and health-related traits. Understanding the genomic basis of divergence among such breeds can provide insights into both evolutionary processes and breeding history. In this study, we investigated genome-wide signatures of selection and genomic differentiation among three autochthonous sheep breeds from Epirus, Greece: Kalarrytiko, Katsika, and Oreino. Genome-wide allele frequency data from 53,274 SNPs and approximately 100 animals per breed were used to detect lineage-specific divergence. Breed-specific differentiation was quantified using the population branch statistic (PBS), while absolute allele frequency differences ($|\Delta p|$) were calculated between each focal breed and the mean allele frequency of the remaining breeds. To reduce stochastic variation at individual loci, both statistics were summarized across non-overlapping windows of ten SNPs. Candidate regions were identified using a stringent empirical threshold corresponding to the top 0.1% of the genome-wide distributions for both PBS and $|\Delta p|$, and adjacent significant windows were merged to define contiguous selection sweeps. Candidate genes were defined as genes whose genomic coordinates overlapped the detected sweep regions according to the Oar_v4.0 reference genome annotation. Several genomic regions showed signals of breed-specific genomic differentiation. A candidate region detected in the Kalarrytiko breed overlapped the LCORL–NCAPG locus on chromosome 6. This region showed elevated $|\Delta p|$ values and clear allele-frequency divergence relative to the other breeds. The LCORL–NCAPG locus has been widely associated with body size and growth traits in livestock, suggesting that variation in this region may contribute to phenotypic differentiation among Epirus sheep breeds. Another candidate region detected in the Oreino breed overlapped the prion gene cluster (PRNP, PRND, PRNT). The PRNP gene (OAR13) encodes the prion protein and is a major determinant of genetic susceptibility to scrapie in sheep. The differentiation signal was concentrated in a narrow cluster of highly divergent SNPs, indicating localized divergence at the PRNP locus. Given that Kalarrytiko and Katsika have been selected for scrapie resistance, whereas Oreino has not, this pattern likely reflects divergence generated by selection in the former two breeds rather than positive selection acting specifically in Oreino. Additional genes overlapping sweep regions included CBS, CRYAA, NDUFV3, PKNOX1, SIK1, U2AF1, RASSF2, SLC23A2, and TMEM230, which are involved in diverse metabolic, regulatory, and cellular processes. Although the functional roles of these genes in sheep production traits remain to be clarified, their presence within differentiated genomic regions suggests potential involvement in breed-specific adaptation. Overall, the results highlight genomic regions shaped by both artificial selection for disease resistance and selection related to growth and physiological processes, providing new insights into the genomic architecture and evolutionary differentiation of indigenous sheep breeds from Epirus, Greece.

Variation in host transcriptomic responses in three SRLV-associated tissues in relation to sheep serological SRLV infection status, TMEM154 E35K polymorphism and breed

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Small ruminant lentiviruses (SRLV) are prevalent pathogens in sheep populations. The TMEM154 E35K polymorphism is associated with SRLV infection risk; sheep carrying the KK genotype can exhibit reduced susceptibility compared to those with EK or EE genotypes. The influence of this polymorphism on the susceptibility of the host varies according to sheep breed and SRLV genotype. This study aimed to analyze transcriptome differences in three tissues targeted by SRLV−spleen (S), lung (L), medulla oblongata (M) from sheep with opposite serological SRLV infection status and different TMEM154 E35K genotypes, belonging to two sheep breeds. Merinoland (ML) and East Friesian Milk (EFM) sheep ewes (≥ 3 years old) were serologically tested for SRLV and genotyped for the TMEM154 E35K variant, resulting in 3 groups: (1) SRLV-positive, TMEM154 EK (posEK); (2) SRLV-positive, TMEM154 KK (posKK); and (3) SRLV-negative, TMEM154 KK (negKK). After RNA sequencing, differentially expressed genes (DEGs) and enrichment analyses were performed. The analyses revealed breed- and tissue-specific transcriptional patterns. In ML sheep, the highest number of DEGs was observed in M (258) and S (265) tissues between posEK and posKK sheep. These DEGs included genes linked to chemokine-mediated signaling pathway and inflammatory response. In S tissue, specific DEGs (EIF2AK2, RC3H2, and RIGI), which play roles in activating antiviral response mechanisms, were enriched in the double-stranded RNA binding term. All of the above-reported GO terms were significant, except the inflammatory response term (FDR = 0.07 after Benjamini–Hochberg (BH) correction). The L tissue showed few DEGs (30) between these groups. Low numbers of DEGs were found across the three tissues in the remaining group comparisons. In EFM sheep, the highest number of DEGs (71) was observed between posKK and negKK sheep in L tissue. These DEGs were enriched for several GO terms, including type I TGF-β receptor binding term. In this tissue, 47 DEGs were found between posEK and posKK sheep, with several genes enriched in extracellular space and inflammatory response terms. In the S tissue, 37 DEGs were observed between posEK and negKK sheep, including three DEGs enriched in defense response to virus term. Moreover, 28 genes were differently expressed between posEK and posKK groups, including 2 genes enriched in the antibacterial humoral response term in S tissue. The above-reported GO terms in EFM sheep reached nominal significance ($p < 0.05$). However, after BH correction, only the extracellular space term remained significant (FDR < 0.05). Few DEGs were found in M tissue (15) between posKK and negKK groups. In conclusion, ML sheep exhibited more pronounced responses in the central nervous system and spleen, whereas EFM sheep demonstrated stronger transcriptional modulation in lung. These tissue differences as well as the differences in DEG numbers and genes between same TMEM154 genotype comparisons in both breeds are in concordance with both the previously observed different SRLV susceptibility and the differences in association of the TMEM154 genotype KK with serological SRLV infection status in the two breeds. Funding: National Science Centre, Poland (UMO-2020/39/I/NZ9/01304), German Research Foundation (DFG, LU 1439/4-1). Bioinformatic analysis: Poznań Supercomputing and Networking Center.

Metabolic stress reshapes intracellular signalling in ovine hypothalamic KNDy neurons

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Energy balance is a key determinant of reproductive physiology in mammals through hypothalamic mechanisms linking metabolic cues with neuroendocrine regulation. Nutrient availability and circulating metabolic signals are integrated by hypothalamic neurons to coordinate reproductive activity with the energetic status of the organism. Among hypothalamic neuronal populations, KNDy neurons located in the arcuate nucleus play a central role in regulating gonadotropin-releasing hormone (GnRH) secretion and reproductive axis activity. Disturbances in lipid metabolism can influence hypothalamic signaling processes involved in this regulatory network. Elevated circulating fatty acids during metabolic imbalance may disrupt intracellular signaling within hypothalamic neuronal networks. Lipid oversupply can alter neuronal metabolism, promote oxidative stress and modify signaling pathways essential for proper hypothalamic function. However, the molecular pathways connecting lipid-induced metabolic stress with hypothalamic signaling remain insufficiently characterized. Experiments were conducted using the immortalized ovine KNDy neuronal cell line FRI273 derived from the fetal sheep mediobasal hypothalamus and kindly provided by Dr. Patrick Chappell (Oregon State University). Cells were cultured in high-glucose Dulbecco's modified Eagle's medium supplemented with 10% FBS and exposed to palmitate (5 mM) for 24 h to induce metabolic stress. Proteomic and phosphoproteomic profiling was performed using multiplexed TMTpro LC-MS/MS. Data analysis was carried out using MaxQuant and Perseus against the UniProt Ovis aries reference proteome. Among the quantified proteins, approximately 15% showed significant abundance changes ($q < 0.05$). Several enzymes involved in neuronal metabolism were increased, including hexokinase-2 (FC 1.08), fructose-bisphosphate aldolase (FC 1.28) and HMG-CoA synthase (FC 2.77). Proteins associated with redox regulation were also elevated, including NAD(P)H dehydrogenase [quinone] 1 (FC 1.19) and heme oxygenase-1 (FC 1.17). Phosphoproteomic analysis identified 4,086 phosphosites, with 18% showing significant changes ($q < 0.05$). Notable phosphorylation changes were detected in proteins involved in neuronal signaling and membrane trafficking, including the GABA receptor alpha-3 subunit (FC −1.14), dynamin-1 (FC −1.22) and clathrin heavy chain (FC −1.23). These results show that metabolic stress affects proteins associated with neuronal metabolism, redox homeostasis and synaptic signaling in ovine hypothalamic neurons. Increased abundance of metabolic and redox-related proteins suggests activation of adaptive metabolic and antioxidant responses to lipid overload. In parallel, altered phosphorylation of proteins regulating membrane trafficking and cytoskeletal organization indicates remodeling of intracellular signaling processes involved in neuronal regulation. Such phosphorylation-dependent changes may influence receptor trafficking and synaptic responsiveness within hypothalamic networks. Together, these findings indicate coordinated metabolic and signaling adaptations of hypothalamic neurons exposed to lipid-induced metabolic stress. Understanding these responses may help explain how metabolic imbalance affects hypothalamic mechanisms regulating reproductive physiology in mammals, including livestock species. This research was financially supported by the National Science Centre, Poland (Grant No. UMO-2023/51/B/NZ9/00651).

Association of C18 fatty acids, including the shift-t10, with the rumen microbiome in lambs

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The rumen microbiota, particularly bacteria, play a crucial role in the biohydrogenation of dietary polyunsaturated fatty acids. This complex process leads to the formation of various intermediates, including trans and conjugated fatty acids, as well as saturated fatty acids, as end products. A phenomenon known as the ”trans-10 shift” occurs when the production of trans-10 biohydrogenation intermediates increases at the expense of trans-11. This shift is believed to be linked to changes in the rumen microbial population. However, there is limited information about the specific microbes responsible for this alteration. This work aims to explore the relationship between C18 fatty acids, particularly the main trans isomers, the 18:0 as the end product, and the trans-10 shift (ratio 18:1t10/18:1t11), and the rumen microbiome composition in lambs. Approximately 150 rumen content samples, previously collected and phenotyped for fatty acid composition, were selected based on high or low trans-10 shifts for 16S rRNA gene sequencing, to identify and classify rumen microorganisms, particularly bacteria, and correlate these findings with C18 fatty acids. Total DNA was extracted from freeze-dried rumen contents using a Qiagen QIAmp DNA Stool Mini Kit (Qiagen Inc., Valencia, CA, USA), according to the manufacturer’s instructions. DNA amplification, library preparation, and sequencing were performed by the MR DNA™ commercial service (Shallowater, TX, USA). Data was analysed using the software R. First, microbial species data were Centred Log-Ratio (CLR) transformed before analysis. Associations between microbial species and fatty acids were evaluated using Spearman correlations followed by Benjamini–Hochberg False Discovery Rate (FDR) correction. Associations with FDR < 0.05 were considered significant. The rumen microorganisms included 97.2% bacteria and 2.7% archaea. Among the composition of bacteria at the phylum level, the most abundant community in the lamb’s rumen was Bacillota (27.6%), Bacteroidota (16.4%), Fibrobacteres (14.7%), Proteobacteria (12.6%), and Bacteroidetes (7.3%). Significant associations were observed between bacterial species and fatty acids related to ruminal biohydrogenation, especially 18:1t10 (802 significant associations) and shift-t10 (761 significant associations). Even 18:1t11 and 18:0 showed 506 and 202 significant associations, respectively. Concerning those associated with 18:1t10, the top 10 included Prevotella lacticifex, Anaerotruncus rubiinfantis, and Butyrivibrio fibrisolvens, some of which are already known bacteria involved in the biohydrogenation of unsaturated fatty acids. These results support the idea that coordinated changes in microbial community composition accompany a shift in biohydrogenation pathways. Integration of these results with genotyping data is ongoing to identify genomic variants linked to resistance to the trans-10 shift. Financial support was provided by PTDC/CAL-ZOO/29654/2017 and UIDB/152816/2022 research projects, UIDB/00276/2020, and LA/P/0059/2020 – AL4Animals.

Assessing the population structure in a recently established hair sheep population using pedigree data

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Sheep breeding in Germany is characterised by a small number of large-scale farms and many smallholders. While smaller farms mainly keep sheep often as a hobby, larger farms rely on a high turnover of lambs and low production costs. This also includes fewer management expenses, such as shearing or treatment costs. In 1998, a group of German sheep breeders and farmers crossed Merino ewes with a Wiltshire Horn ram to create a new breed of sheep. The aim of the breeding programme was to produce a robust, polled hair sheep that did not require shearing, but which showed good carcass characteristics. Already after a few generations, most lambs showed preferable traits, especially self-shedding of hair. Further on the mating scheme included Barbados Blackbelly, Berrichon du Cher, Cameroon and Dorper rams for additional improvement. Following the successful development of a robust hair sheep, the population was divided into two distinct breeds based on appearance: the meat-focused Nolana sheep and the Brown sheep, which is better suited to landscape conservation. While these breeds are relatively young, the limited initial population size may increase the risk of inbreeding. This study aimed therefore to determine the population structure of Nolana sheep and establish the risk of inbreeding within and across farms. Pedigree data from more than 17,000 Nolana sheep were investigated using the OptiSel package in R (version 2024.12.0 + 467). With an effective population size exceeding 50, this breed is, by definition, not at risk. Furthermore, there is a growing interest in the breed, accompanied by an increase in population size. The average inbreeding coefficient was low (F = 3.9%), though an increase was observed on some farms. Inbreeding coefficients were similar in ewes and rams. Most of the Nolana sheep investigated had an inbreeding coefficient below 1%, with nearly half of the population showing no inbreeding. While an increase of the inbreeding coefficient was observed until 2010, recent years showed a decline again. An average kinship coefficient between pKin = 1 % and 3 % was observed. The analysis of the genetic contribution showed the larger contribution of Wiltshire Horn and Dorper rams, and smaller contribution of Cameroon and Barbados Blackbelly rams. The farm-based analyses showed larger management differences based on the introduction of other breeds, this especially contributed to the aim for an improved carcass quality. Based on the population size and inbreeding, this study indicates that the Nolana breed is currently not at risk. Genomic analysis could help to improve our understanding of the population structure and breeding programme.

Rethinking phytate utilisation in poultry: Endogenous phytate degradation and influencing factors

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Phytate-bound phosphorus (P) in plant feedstuffs was long considered unavailable to poultry. This concept shaped feeding strategies, P evaluation assays, and development of exogenous enzymes. However, poultry possess an endogenous capacity to degrade inositol-hexakisphosphate (InsP6), releasing P and myo-inositol (MI). This contribution summarises recent findings on endogenous InsP6 degradation in growing birds and laying hens. In broiler chickens, endogenous InsP6 degradation can exceed 70% and responds strongly to dietary mineral P and calcium (Ca). Mineral P, and particularly excess Ca, reduces InsP6-P digestibility. Therefore, P digestibility values of individual feedstuffs are not additive in mixed diets. In broilers, changes in InsP6 degradation are consistently associated with alterations in ileal and plasma MI and P digestibility. In young turkeys, lower degradation than in broilers was observed when species-specific diets were fed. When both species received the same diet, mucosal phytase activity, InsP6 degradation, and P digestibility did not differ at 3 weeks of age, indicating similar intrinsic capacities. At 6 weeks, turkeys showed higher enzyme activity. MI responses differed markedly between species, suggesting species-specific MI metabolism. In laying hens, the interdisciplinary Research Unit "P-Fowl" (https://p-fowl.uni-hohenheim.de/en) found that Ca in feed and intestinal lumen is a stronger regulator of endogenous InsP6 degradation than dietary mineral P. Complete renunciation of mineral P only modestly stimulated degradation under high-Ca conditions. This contrasts with broilers and turkeys. A marked physiological shift occurs at the onset of laying. The transition from pre-lay (~19 weeks) to active lay (~24 weeks) represented a comprehensive systemic adaptation of mineral metabolism. After onset of lay, mucosal phytase activity and InsP6 degradation increased, accompanied by changes in intestinal gene expression, endocrine regulation, plasma mineral profiles, transporter abundance, and medullary bone. Genetic background introduces further variability. Lohmann Brown (LB) and Lohmann Selected Leghorn (LSL) hens showed distinct metabolic strategies in the P-Fowl studies. LB exhibited higher mucosal enzyme activity, higher intestinal InsP-related gene expression, higher plasma phosphate and MI, and greater ileal MI flow and disappearance. LSL but not LB showed increased intestinal sodium-dependent phosphate transporter (NaPi-IIb) protein expression at onset of lay. With graded MI supplementation, LB upregulated NaPi-IIb, whereas LSL downregulated it, without major plasma P changes. MI is not merely a degradation end-product but a metabolically active molecule linking InsP6 hydrolysis to intracellular phosphate sensing and energy homeostasis via inositol phosphates and pyrophosphates. LB appear to have a metabolic strategy with enhanced MI turnover and phosphate transport, associated with higher egg MI concentration. LB showed greater quantitative genetic variation in P metabolism-related traits. Heritability of InsP6 degradation and plasma MI was found only in LB. Overall, poultry show high endogenous potential for InsP6 degradation but marked differences in adaptation. In growing birds, mineral P supplementation is a primary cause of reduced endogenous degradation, whereas in laying hens, high Ca supply predominates. This complexity challenges P evaluation systems. Endogenous InsP6 degradation is central to a system spanning the intestine, bone, endocrine axes, and involving the animal genome, transcriptome, and metabolome.

Dynamics of endocrine and transcriptional profiles in phosphorus and calcium metabolism at onset of egg laying

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During sexual maturation in female chickens, endocrine factors such as estradiol, PTH and vitamin D regulate the transition from haematopoietic to medullary bone and coordinate calcium (Ca) and phosphorus (P) homeostasis. Medullary bone forms within the cavities of long bones and serves as a mobilizable Ca source for eggshell mineralization. Adaptive changes in renal reabsorption and systemic mineral metabolism maintain mineral homeostasis at high daily Ca turnover at onset of egg laying. During maturation, laying hens need to manage sharply elevated Ca demands for eggshell formation while forming medullary bone, preserving skeletal integrity and mineral homeostasis. In this study, Lohmann Selected Leghorn (LSL) and Lohmann Brown (LB) were fed sequential developer, pre-layer, and layer diets according to current recommendations. Plasma, kidney, and femur samples were collected from weeks 16-24, covering the pullet stage to onset of lay. Plasma metabolites and gene expression of medullary bone and kidney were analysed. The temporal profile during the study period showed an increase in serum β-CTX-1 in weeks 17 to 21, which was attributable to early matrix degradation, an increase in estradiol in weeks 20 to 21, and the onset of Ca and P accumulation in the medullary bone in weeks 22 to 23. Renal expression of genes involved in active mineral transport (SLC34A1, TRPV6) was low in immature birds until ~20 weeks of age and an upregulation towards week 23. Genes involved in tight junction formation (CLDN10, OCLN) were lowered in mRNA abundance in immature pullets, with lowest values around week 20. Expression of receptors of endocrine regulators (PTH2R, VDR) suggested reduced renal sensitivity to PTH and vitamin D3 in response to high Ca intake along sexual maturation, which might promote hypercalciuria and phosphaturia in the period prior onset of egg laying. Around week 20/21 of life, endocrine cues triggered renal adjustments maintaining hypercalcemia to initiate eggshell formation. Along maturation, the corresponding gene expression profiles in medullary bone indicated synchronized regulation of vascular, metabolic, and osteogenic pathways, with the overall remodelling process commencing earlier in LSL than in LB hens. Deconvolution of bulk mRNA sequencing data estimated medullary bone cell composition. Erythroid lineage cells constituted about 85% within the bone cavity, whereas mesenchymal cells, including osteoblasts, accounted for only 0.33%. During maturation, macrophage and lymphatic cell populations expanded at the expense of erythroid cells. Osteoblasts were absent in both strains at week 19 but were detectable by week 24, with higher proportions in LSL than LB. Overall, successful medullary bone formation and mineral metabolism in the kidneys occurred in a cascade-like sequence in both LB and LSL laying hen strains. Analyses identified key genes and pathways for genetic or nutritional interventions aimed at maintaining efficient mineral turnover and mineral use efficiency throughout the laying period. Funding: This work was supported by the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) – Project number 322916021 and was part of the Research Unit FOR 2601: Inositol phosphates and myo–inositol in the domestic fowl: Exploring the interface of genetics, physiology, microbiome, and nutrition.

Strain-Specific Molecular, Immune, Physiological, and Microbiome Shifts Along the Gut–Bone–Kidney Axis Under Mineral Phosphorus Depletion in Laying Hens

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The maturation of laying hens offers a powerful model to study how phosphorus (P) homeostasis is regulated along the gut–bone–kidney axis. Under low P supply, endocrine factors such as vitamin D□ increase intestinal mineral absorption and renal mineral reabsorption. Moreover, the turn-over of minerals in the medullary bone needs to be ensured for eggshell formation. The aim of this study was to integrate multi-omics data to uncover production-stage-dependent molecular and physiological changes along the gut-bone-kidney axis in two laying hen strains fed diets with or without mineral P supplementation. Briefly, 80 laying hens (40 Lohman Brown (LB), 40 Lohman Selected Leghorn (LSL)) were used in a family-balanced 2×2×2 factorial design testing strain, diet (P+ supplied with +1 g/kg non-phytate P vs. P− without mineral P), and age (19 vs. 24 weeks (wk)). We collected plasma, jejunal mucosa, kidney, medullary femur bone, duodenal digesta, and excreta, and measured immune traits in blood, spleen, and cecal tonsils. After standard quality control and data-type-specific normalization/transformation, we applied the DIABLO framework in mixOmics (block sPLS-DA) to integrate the datasets (biochemistry of gut and blood, microbiota, immune traits, bone and body phenotypes, epigenetic layers (miRNA, ATAC-seq), and mRNA-seq from gut/bone/kidney) and identify multi-omic biosignatures discriminating strain and dietary P at 19 and 24 weeks across three latent components. Component 1 mainly captured the age (24 vs 19 wk), separating 24-wk from 19-wk hens across strains and diets. The wk 24 hens showed higher mRNA signals across bone (VLDLR/GREB1L), gut (SLC30A10/EPHA4/KCNK17), and kidney (ASIC4/HSD17B2), a shift toward innate and mucosal immunity, intensified mineral handling (plasma Ca/estradiol), higher gut myo-inositol and excreta P, enriched Lactobacillaceae ASVs (Amplicon Sequence Variants), and altered femur traits. In contrast, week 19 hens displayed higher abundance of certain miRNAs, stronger blood T-cell/lymphocyte signatures, elevated plasma β-CTX, T3, inorganic P, higher excreta Ca, and increased jejunal promoter accessibility at immune/remodeling loci. Component 2 reflected a strong strain axis, linking LSL to jejunal CHST14/HCK expression, open lncRNA/rRNA promoter regions and miR-7460-3p, Lactobacillus/Limosilactobacillus enrichment, and T-cell–related immunity. In contrast, LB showed higher kidney MYL1/CD200L/PRDM8 and bone MHC-Y–related expression, higher Lacticaseibacillus/Helicobacter abundances, higher plasma calcidiol, higher plasma IgA and heterophils, greater intestinal InsP6/ileal myo-inositol with increased P excretion, and larger femurs and immune organs. Component 3 captured the dietary P effect (P− vs P+; clearer at 19 than 24 week), with coordinated shifts in gut/kidney/bone transcripts (ECHDC3/CLDN14/PASSS2), miRNAs/ATAC changes, Lactobacillaceae enrichment (mainly Ligilactobacillus ASVs), innate/mucosal markers (plasma monocytes/heterophils and IgA) and matching changes in gut chemistry, mineral-endocrine markers (Pi, PTH/Mg) and bone/body traits. Overall, the integrative framework distilled multidimensional datasets into interpretable biosignatures to separate the effects of age, strain, and diet across multiple data blocks simultaneously.

Quantitative genetic insights into calcium and phosphorus metabolism at the peak of egg laying of two commercial laying hen strains

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Phosphorus (P) and calcium (Ca) are essential minerals for laying hens. Phosphorus is primarily bound as phytate in plant feedstuffs and P availability is therefore dependent on the enzymatic activity of phytase. Due to the high Ca intake of laying hens, intestinal phytate hydrolysis is limited, thereby reducing the availability of P. As P and Ca metabolisms are complex and interdependent, the present study investigated traits of P and Ca metabolism in two commercial laying hen strains at the phenotypic and quantitative genetic level. By using phenotypes obtained from different tissues relevant to these metabolic pathways, as well as important performance traits, a comprehensive picture of P and Ca metabolism was generated to get in-depth insights into the role of genetics. The experimental population comprised 200 Lohman Selected Leghorn (LSL) and 200 Lohman Brown (LB) hens that descended from 10 unrelated roosters per strain. Genotyping was performed using 60K SNP chips. All hens were hatched and reared under the same standardized conditions. During experimental periods in the subsequent laying phase, the hens were kept in individual metabolic units and fed the same P-reduced diet, aiming at a low P supply. Sampling was conducted across four consecutive cohorts at 27, 31, 35, and 39 weeks of age, respectively. Depending on the cohort to which they belonged, the animals were 29, 33, 37, and 41 weeks old at excreta sampling and 30, 34, 38, and 42 weeks old at slaughter. An even distribution of the strains and offspring per rooster across cohorts was ensured. Excreta, eggs, blood plasma, and ileal digesta were sampled, either in live animals after a habituation phase or at slaughter. Laying performance, feed intake, and body weight were continuously measured. Traits of P and Ca metabolism were derived from the samples, including concentrations and retention of P and Ca, as well as myo-inositol concentrations. Population genetic analyses of the two strains revealed large differences between LSL and LB; hence all subsequent analyses were conducted separately for each strain. Mixed linear models were applied to assess additive genetic variation and calculate heritability for each trait. Correlations between traits were calculated from phenotypes that were pre-corrected for systematic effects. Most of the traits differed between the strains at the phenotypic and quantitative genetic levels. Whereas the heritability of P and Ca concentration in excreta, plasma and ileal digesta as well as their retention in the excreta was small to moderate (0.03 to 0.27), heritability of myo-inositol concentration was significant in eggs ($h^2 = 0.69$ in LSL, $h^2 = 0.55$ in LB), blood plasma ($h^2 = 0.43$ in LSL, $h^2 = 0.36$ in LB), and ileal digesta ($h^2 = 0.60$ in LB). The correlation showed that the general relationship between traits might be comparable across the strains. Overall, the study provides insight into the genetic determination of traits reflecting phytate degradation and P and Ca utilization in commercial layers at peak lay.

Omics-based covariance matrices in prediction models: a case study phosphorus efficiency and poultry

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A central aspect of quantitative genetics is the prediction of complex quantitative traits. Since the use of SNPs has greatly improved breeding value estimation, new omics-based technologies are now available that are inexpensive, guarantee high output, and ideally bridge the gap between animal DNA and the phenotypic expression of quantitative traits. Today, studies increasingly use specific transcriptome or microbial data, often derived from target tissues or specific sections of the gastrointestinal tract. Typically, classical covariance matrices are used in best linear unbiased prediction (BLUP) models incorporating transcriptomic or microbial data as explanatory variables; here is an matrix containing the z-standardized molecular feature values, where is the number of animals and the numbers of molecular features (e.g., microbial taxa abundances or transcript expression levels). In this context, there is often discussion about using covariance matrices that better reflect the biological structure of the data, i.e., covariance matrices that are calculated based on distances, dissimilarities, or ordinations rather than simple similarities. This is expected to result in a better representation of reality and is predicted to lead to higher proportions of explained phenotypic variance and greater accuracy in trait predictions. To verify whether we can truly gain added value by using alternative omics-based covariance matrices, we tested different methods for calculating covariance matrices on a data set of a Japanese quail F2 cross phenotyped for phosphorus efficiency and related efficiency traits. The birds were genotyped, and ileum miRNA gene expression and ileum microbiota composition were classified. The data set contained 760 quail with 4k SNP genotypes, known microbial composition with 1,188 OTUs, and trait records and 481 quail with additional 77 miRNA gene transcript abundances. We tested different dissimilarity (e.g., Euclidean distance, Jaccard distance) and ordination measurements (e.g., redundancy analysis, multidimensional scaling) to build a similarity matrix and compared it with the classical covariance matrix. These matrices were compared using several BLUP models containing the microbial information (MBLUP), the transcriptomic information (TBLUP), and models containing genomic plus microbial or transcriptomic information in one model (GMBLUP and GTBLUP). At first glance, it appears that we can explain up to 30% more phenotypic variance for microbial and up to 19% more for transcriptional data using alternative covariance matrices compared to the classical matrix. However, these explained variances turn out to be merely artifacts when considering phenotypic trait predictions, the significance tests for them, and the mean square errors of the phenotypic predictions. No added value for phenotypic trait predictions was found when using alternative covariance matrices that utilized either microbial or transcriptional data. Depending on the method used, the accuracies of trait predictions are at a similar level or even significantly lower, despite considerably greater explained variance. For this reason, we consider the use of alternative covariance matrices, which are intended to better reflect the biological structure of molecular features, to be unjustified.

The importance of minerals and vitamins for poultry health

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The importance of vitamins and trace elements in immune defence has long been the focus of scientific research, with the consequences of inadequate or inappropriate intake being widely discussed topics. This keynote presentation will illustrate the importance of these nutrients using a few examples from poultry farming. Certain micronutrients work synergistically to support the various components of the immune system, such as physical barriers, cellular responses and antibody production. Insufficient or deficient levels of these micronutrients negatively affect the body's defences, impairing its ability to fight infections. A number of trace elements influence the functions, processes and mechanisms of the immune system. Therefore, an inadequate supply also leads to reduced resistance to infections. In this context, selenium, copper and zinc are of particular interest and have been frequently studied. The prime antioxidant components in poultry feed are vitamin E, selenium and carotenoids. The synergistic effect of selenium and vitamin E on immune system function is well documented and widely recognised. While both nutrients perform independent functions in the body, they both play a vital role in the antioxidant system. Selenium is an essential component of the glutathione peroxidase enzyme family. Vitamin E is a vital fat-soluble antioxidant that protects against lipid peroxidation caused by free radicals. Copper is involved in both humoral and cell-mediated immunity. Its role in the immune system is linked to the function of the antioxidant system, as it is an essential component of both copper-zinc dismutase and ceruloplasmin, defending living organisms against reactive oxygen species. However, copper acts as a pro-oxidant when it is unbound. For example, Zn contributes to the maintenance of immune function, growth performance and skeletal development in broiler chickens. Severe zinc deficiency significantly impairs immune system function in animals. However, a slight zinc deficiency has no effect on immune system function. Vitamins are essential nutrients required for optimum general health and physiological functions, such as development, growth, maintenance and reproduction. Most birds cannot synthesise vitamins and must therefore obtain them from their feed. However, feed alone is not sufficient to cover vitamin requirements. Vitamin-supplemented diets play an important role in disease treatment and prevention. For instance, vitamin E is a biological antioxidant that improves the growth performance and the physiological and immunological status of broiler chickens by reducing lipid peroxidation and neutralising free radicals in skeletal muscle and plasma. Vitamin D3 is important for bone growth and plays a critical role in biological pathways such as immune function, calcium homeostasis, and cellular proliferation and differentiation. Vitamin A is necessary for visual development, growth, reproductive physiology and maintaining the integrity of epithelia and the skeleton. It also supports an optimum immune response, thus diminishing susceptibility to infection. Trace elements and vitamins are therefore crucial nutritional components for poultry. Issues of stability (e.g. vitamin A) and formulation (e.g. organically bound trace elements) are of particular interest in this context.

Impact of feeding diets without mineral P supplement on the immune system of two laying hen strains

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Phosphorus (P) is an essential nutrient for tissue development, cell function and biochemical signaling pathways. An adequate P availability is also a fundamental prerequisite for an efficient and well-regulated immune system. In the context of sustainable animal husbandry, reducing mineral P in feed is becoming increasingly important. Recent studies indicate that mineral P supplementation in poultry diets can be substantially reduced, as poultry can utilize much of the phytate-bound-P in plant feedstuffs when mineral P levels are kept low. Moreover, research shows that reducing mineral P in laying hen diets does not adversely affect the immune system (Hofmann et al., 2021, doi: 10.3390/ani11010129). Therefore, the objective of this research was to investigate the effects of a complete renunciation of dietary mineral P on the peripheral and gut-associated immune system of two laying hen strains. The current study is part of interdisciplinary research project P-FOWL, which investigates the influence of inositol phosphates and myo-inositol at the interfaces of genetics, physiology, microbiome, and nutrition in domestic chickens (https://p-fowl.uni-hohenheim.de/). The experiment described here was based on a 2 × 2 × 2 factorial design (P × laying hen strain × week of life). Feed rations were either enriched with 1 g/kg mineral P (P+) or contained no mineral P supplement (P-) and were fed for a total of 4 weeks (wk). Lohmann Brown Classic (LB) and Lohmann LSL Classic (LSL) hens were sampled at the age of 19 or 24 wk to additionally investigate the impact of the onset of egg laying. Absolute numbers of leukocyte subsets in blood, spleen and cecal tonsils (CT) were measured using flow cytometry. Antibody concentrations in blood plasma and bile were analyzed by ELISA. Mitogen-induced splenocyte proliferation was detected using a lymphocyte proliferation assay and gene expression of pro- and anti-inflammatory cytokines were analyzed by qRT-PCR in the spleen and CT. Statistical analysis was performed using SAS 9.4 software using a linear mixed model. Statistical significance was set at $P < 0.05$. The complete renunciation of dietary mineral P affected the systemic and gut-associated immune system of LB and LSL hens. The results show that the cytokine gene expression and the distribution of immune cell populations were influenced by the absence of mineral P supplementation in the feed. In the CT, the cytokine profile of hens fed the P- diet was characterized by high expression of IL-2 and IL-10 and by higher proportions of NK cells and $CD4^{+}CD25^{high}$ T cells. The latter are described to represent regulatory T cells, which increased proportion corresponds to higher expression of IL-10. Together, this could indicate an increased immunoregulatory activity and anti-inflammatory immune status in the hens fed P- diets. Besides, the P- diet led to lower immune cell counts in the blood, including total leukocytes, total T cells and $CD4^{+}$ T cells. In this context, a targeted regulation of the activation of pro-inflammatory T cells in the CT could be a possible cause for the decline in immune cell populations in the blood when fed the P- diet. Overall, our findings suggest that the renunciation of mineral P supplementation modifies immune regulation within the gut-associated immune system and fosters an anti-inflammatory condition. The immunological changes associated with renunciation of mineral P supplementation cannot yet be conclusively evaluated as beneficial or detrimental; further studies, including infection trials and prolonged withdrawal of dietary mineral P, are required.

Impact of myo-inositol feeding and mineral phosphorus supply on insulin signaling in liver and adipose tissue of two high-performing laying hen strains at different production stages

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As in other species, insulin plays a major role in energy and protein homeostasis of chicken, too. Insulin action might change throughout the production cycle of laying hens due to the changing nutrient and metabolic demands. Myo-inositol (MI) is discussed to modulate insulin signaling cascade (ISC), at least in broilers. For various cellular signaling processes depend on phosphate, dietary supply of mineral phosphorus (P) might also affect ISC. However, P utilization and MI metabolism differ between certain layer strains suggesting variations in ISC response to dietary MI or P. Thus, the present study aimed to assess the impact of different concentrations of MI or omission of mineral P in the diet on hepatic and adipose ISC of Lohmann Brown (LB) and Lohmann Selected Leghorn (LSL) hens at certain stages of production cycle. The study involved two trials. Trial 1 comprised 40 LB and 40 LSL hens, divided into four feeding groups supplied with 0 (control), 1, 2 or 3 g of MI per kg feed. After 4 weeks (wks), hens were slaughtered at 30 wks of age (laying peak). Trial 2 comprised 40 LB and 40 LSL hens that were fed either 0 (P_-) or 1 g/kg (P_+) of mineral P 4 wks prior to slaughter. Half of these hens were either slaughtered at 19 wks (before onset of egg laying) or at 24 wks of age (laying phase). Protein abundance of phosphoinositide 3-kinase (PI3K), mammalian target of rapamycin (mTOR), insulin receptor β (INSR), protein kinase B (PKB), adenosin monophosphate activated kinase (AMPK) and their phosphorylated forms (pmTOR, pINSR, pPKB and pAMPK) was assessed in liver and abdominal adipose tissue by Western Blot. Statistical analysis used a general linear mixed model with the fixed factors strain and diet for Trial 1 and strain, diet and production stage for Trial 2. In Trial 1, the most prominent effect was exerted by strain with higher abundance of hepatic INSR, pINSR, pmTOR, pAMPK and adipose pmTOR in LB than LSL. In both tissues effects of strain and diet mainly interacted. For example, in MI-supplemented groups AMPK in LB was higher expressed than in the control group whereas in LSL it was lower than in the control group. In Trial 2 production stage exerted the most prominent effect in both tissues. Hepatic INSR, pINSR, PI3K and AMPK were higher expressed at wk 19 than wk 24. This difference was more distinct in LSL compared to LB. Further LSL hens had higher expressions of pPKB in adipose tissue at wk 24 than LB hens at that age. As in Trial 1, effects of diet and strain mainly interacted, leading partially to opposite effects in LB and LSL associated with mineral P supply. For example, LB P_+ hens showed lower abundance of PI3K and AMPK in liver than LB P_- hens, but in LSL P_+ hens abundance of PI3K and AMPK was higher than in LB P_- hens. hens The present study implies that strain and production stage might have a stronger impact on insulin signaling in laying hens than MI feeding or omission of dietary mineral P. Results from Trial 1 suggest enhanced activation of insulin signaling in favor of protein synthesis in LB hens at laying peak. Differences in ISC between hens before and after onset of egg laying might reflect nutrient partitioning in support of egg production. However, interactions of diet and strain may indicate that differences in terms of P and MI metabolism also subsequently affect ISC dependent metabolism.

Risk assessment of environmental samples on the prevalence of antibiotic-resistant microorganisms in broiler fattening systems

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While the prevalence of antibiotic-resistant microorganisms in the environment poses a risk for human and veterinary medicine, broiler fattening systems might act as a risk factor due to the stagnating high amount of antibiotics used. However, due to potential co-selection of antibiotics and heavy metals via co- and cross-resistance, supplementation of heavy metals as trace elements in amounts above broilers' requirements might increase those risks. Therefore, a risk assessment was conducted to evaluate the effects of the nutritional composition of environmental samples on the occurrence of antibiotic-resistant microorganisms. Environmental samples of broiler farm-related inputs (feed, unused bedding material, water), system-integrated factors (dust) and outputs (litter as bedding and broilers' excreta) were collected during the last week of fattening of 14 commercial, German broiler farms. Feed, bedding and manure were analysed for their nutritional composition (dry matter, crude ash, crude protein, metabolic nitrogen, crude fat, fibre), while elements were measured in all samples. Additionally, the presence of methicillin-resistant Staphylococcus aureus (MRSA) and species with resistance to extended-spectrum beta-lactams was microbiologically detected, further identified via MALDI-TOF MS and tested for antibiotic susceptibility. The accumulation of nutrients and elements was statistically evaluated using an ART model, with sample type as a fixed factor and farm ID as a block structure. Risk assessment was then conducted via Firth's penelized logistic regression, in addition to Pearson's correlation. Both nutrients and elements showed significant accumulation from inputs (feed, bedding, water) to dust, serving as a system-integrated factor, and to litter as an output. Furthermore, especially trace elements in feed (e.g. Cu, Fe, Zn) showed concentrations up to 5 times (for Cu) or 3.5 times (for Fe and Zn) above the recommended levels for broiler nutrition. Additionally, considering Pearson's correlations, relationships between trace elements within one sample type and between different sample types could be detected. In particular, correlations between feed, litter, and dust illustrate the effect of a general supplementation above broilers' requirements. The risk assessment indicated a higher risk for antibiotic-resistant microorganisms when bedding or litter showed high concentrations of crude protein (e.g., for the occurrence of multidrug-resistant microorganisms (MDR) in general or Enterobacteriaceae, $p = 0.008$) or metabolic nitrogen (relative to total nitrogen) (e.g. for the occurrence of MDR in general or Enterobacteriaceae, $p = 0.062$ or $p = 0.043$, respectively). Fibre components in bedding and litter seemed to reduce those risks (e.g., neutral detergent fibre in litter for Enterobacteriaceae, $p = 0.029$). Nutritional factors in feed had a minor effect on the occurrence of antibiotic-resistant microorganisms. Overall, the composition of environmental factors, such as bedding and thus litter, appears to be mainly influential for the appearance of antibiotic-resistant microorganisms. Therefore, when evaluating or choosing bedding materials, one should primarily focus on high fibre concentrations. This work (Model- and Demonstration Project for animal welfare) is financially supported by the Federal Ministry ofFood and Agriculture, granted by the Federal Office for Agriculture and Food.

Effect of varying dietary methionine plus cysteine concentrations on growth performance of slow-growing broiler chickens at different ages

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Slow-growing broiler chickens are increasingly used in alternative production systems in Europe. Due to their lower growth rate, required dietary methionine (Met) plus Cysteine (Cys) concentrations for optimal growth performance may differ from those of fast-growing broiler chicken strains. This study evaluated the effects of varying dietary Met+Cys concentrations on growth performance of male slow-growing Ranger Classic broiler chickens. Two dose–response trials were conducted for feeding phases Grower 1 (PG1, day 7-18) and Grower 2 (PG2, day 18-35) using male Ranger Classic broiler chickens in a randomized block design with six dietary Met+Cys concentrations and five pens per treatment (40 birds/pen). In each phase, the basal diets contained 2.7 g Met/kg and 3.1 g Cys/kg. Graded levels of DL-Met (g/kg diet) were added to the other diets to achieve higher dietary Met+Cys concentrations: PG1: 0.4–2.9; PG2 0.4–3.1. All other nutrients met recommendations to ensure Met+Cys as the first-limiting amino acids. Birds were housed in floor pens and had ad libitum access to feed and water. Body weight and feed intake were recorded to calculate average daily gain (ADG) and feed conversion ratio (FCR). Data were analyzed by one-way ANOVA ($p \leq 0.05$). Further, nutrient–response relationships between daily Met+Cys intake and ADG or FCR were modeled using an interpretable single-neuron machine-learning approach (Ahmadi and Rodehutscord, 2025). Optimal intake was defined as 95% of maximal ADG and 105% of minimal FCR. Dietary Met+Cys concentrations corresponding to these intakes were modelled from the relationship of Met+Cys intake and dietary concentration. Analyzed diets confirmed calculated nutrient concentrations. ADG increased and FCR decreased nonlinearly with increasing dietary Met+Cys, reaching plateaus at 6.6 and 6.9 g Met+Cys/kg for PG1 and PG2, respectively. Maximal ADG was 36.8 g/bird (PG1) and 70.8 g/bird (PG2), and minimal FCR was 1.41 kg/kg (PG1) and 1.57 kg/kg (PG2). Model fit was high for both ADG (PG1 and PG2: $R^2 = 0.96$) and FCR (PG1: $R^2 = 0.92$; PG2: $R^2 = 0.88$). In PG1, optimal daily Met+Cys intake ranged from 341 (ADG) to 310 (FCR) mg/bird, corresponding to dietary Met+Cys concentrations of 6.81 and 6.40 g/kg. In PG2, optimal daily Met+Cys intake ranged from 735 (ADG) to 670 (FCR) mg/bird, corresponding to dietary Met+Cys concentrations of 6.60 and 6.26 g/kg. These optimal dietary Met+Cys concentrations are substantially lower than those recommended for fast-growing broiler chickens that can be found to range between 10.0 and 8.2 g Met+Cys/kg from starter to finisher diets (on a digestible amino acid basis, Aviagen, 2022). Although the use of slow-growing broiler chickens is generally associated with lower resource efficiency, the present findings indicate that optimizing dietary Met+Cys supply to strain-specific requirements may improve amino acid utilization efficiency, reduce nitrogen excretion, and thereby enhance the sustainability of such production systems. Ahmadi, H., Rodehutscord, M, 2025: Nutrient–response modeling with a single and interpretable artificial neuron. Sci Rep 15
Aviagen, 2022: Ross nutrition specifications. Midlothian, United Kingdom

Random forest prediction of nitrogen utilization efficiency in broiler chickens with growth performance and dietary nutrient concentrations

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As the experimental determination of nitrogen utilization efficiency (NUE; percent of N accretion per N intake) is restricted, an accurate statistical method to predict NUE is needed. Compared to linear models, the random forest algorithm has recently been suggested to predict NUE in ruminants (doi 10.1016/j.anifeedsci.2023.115670). Therefore, this contribution evaluated the use of growth performance and diet characteristics to predict NUE of broiler chickens using a random forest algorithm. It was hypothesized that adding dietary amino acids (AA) concentrations as predictors increases the predictability compared to models without AA. A dataset of 528 data lines was initially extracted from 90 peer-reviewed nutrient balance studies from 2016 to 2024 after filtering out data lines where growth performance was reduced by interventions. The AA profile was recalculated from feeding ingredients using the INRAE database for all data lines to avoid missing values. Studies where ingredients were not available in the database were excluded. The final dataset consisted of 371 data lines with no missing values. Five groups of predictors were defined. General accessible predictors (GAP) included dietary crude protein, metabolizable energy, age, feed intake, and weight gain. Further four groups of predictors were GAP plus AA, herein denoted in the three-letter code: first-limiting AA (Gl: Met, Lys, Thr, Trp, Val, Ile), essential AA (Ge: Lys, Thr, Met, Cys, Trp, Ile, Val, Leu, Phe, His, Arg), nonessential AA (Gne: Tyr, Ala, Asp, Glu, Gly, Ser, Pro, Gly equivalent), and all AA (Ga). The five predictor groups were GAP and GAP plus each group of AA, and the dependent variable was NUE. Two Monte-Carlo cross-validation simulations were implemented with the split methods: (I) random split; (II) split by trial to avoid shared trial information between the sets. The number of trees was firstly determined as 1000 by the R package "optRF", and random forest models were computed by the "ranger" package. Both simulations went through 1000 iterations, where models were trained with the training data (70%) and applied to the testing data (30%) for prediction. The goodness-of-fit between predicted and observed NUE was evaluated by root mean squared error (RMSE), mean average error (MAE), and R^2. The concordance to 45° line was indicated by concordance correlation coefficient (CCC). The indicators from 1000 iterations were collected for descriptive analysis. In random split, the inclusion of AA as predictors improved goodness-of-fit and concordance (GAP: RMSE = 5.4, MAE = 3.6; Ga: RMSE = 4.7, MAE = 3.1; GAP: R^2 = 0.58, CCC = 0.70; Ga: R^2 = 0.69, CCC = 0.79). Performances of other AA combinations were similar, e.g., RMSE: Ge = 4.73, Gne = 4.73, Gl = 4.83; CCC: Ge = 0.79, Gne = 0.78, Gl = 0.77. Goodness-of-fit greatly decreased as reduced performances for all models when the data were split by trial compared to random split, e.g., GAP RMSE: 9.35 vs. 5.42, respectively; Ga RMSE: 8.54 vs. 4.66, respectively. These results showed that trial-level information was essential for higher prediction accuracy. Compared to the model without AA, the inclusion of AA improved goodness-of-fit and concordance of the NUE prediction. This effect was influenced by the number of amino acids, but not related to the physiological importance of the AA.

Metagenomic analysis reveals strain-specific ileal microbiome responses to low-phosphorus diets in laying hens

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Improving phosphorus (P) utilization in poultry is critical due to the depletion of global mineral phosphate reserves. While the gut microbiome may contribute to phytate degradation, the interacting effects of host genetics and dietary P restriction on microbial composition remain underexplored. This study investigated the ileal microbiome of two high-yielding laying hen strains (Lohmann Brown Classic, LB; Lohmann LSL Classic, LSL) under low-P supplementation to identify strain-specific microbial and functional adaptations. A total of 400 hens were fed low-P diets, while 40 hens received a P-adequate control diet. After three weeks, ileal digesta were analyzed using shotgun metagenomics. LB hens fed a low-P diet showed significantly higher Shannon diversity than LSL hens fed either a low-P diet or an adequate-P diet. PCoA based on a weighted variant of Bray-Curtis distance metrics showed a significant difference in the bacterial communities of ileal microbiota between two strains, indicating a strong host genetic effect on the ileal microbiota. Differential abundant species analysis showed that the low-P diet shifted the differentially abundant species between LB and LSL hens. At the species level, Ligilactobacillus salivarius and Enterococcus cecorum were more abundant in LB hens, whereas Limosilactobacillus vaginalis and Lactobacillus amylovorus were enriched in LSL hens. Correlation analysis demonstrated that in LB hens, L. salivarius and E. cecorum correlated positively with plasma P concentrations. Conversely, in LSL hens, Limosilactobacillus reuteri and Limosilactobacillus oris correlated positively with plasma P, Ca, and myo-inositol concentrations. Functional profiling using the KEGG database showed that four pathways related to phosphorus metabolism (ABC transporters, mineral absorption, endocrine and other factor-regulated calcium reabsorption, and calcium signaling pathway) were significantly enriched in LSL hens, and three KEGG pathways (phosphotransferase system, inositol phosphate metabolism, and phosphatidylinositol signaling system) were abundant in LB hens. Furthermore, the low-P diet modulated taxa associated with phosphate transport and phytate hydrolysis (including L. salivarius and E. cecorum) in LB hens, while genes encoding phosphonate transport were more abundant in LSL hens. These functional shifts indicated distinct strain-dependent strategies in phosphate transport and inositol-related metabolism under restriction, highlighting the microbiome as a potential target for improving phosphorus efficiency in layer production.

Defatted and dehydrated avocado seed meal improves feed conversion ratio in broiler chickens at 21 days of age

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Alternative feed ingredients in broiler diets require the definition of safe inclusion levels. Avocado seed contains fibrous fractions and phenolic compounds; however, information regarding its use in broiler diets remains limited. This study evaluated the effects of dietary inclusion of defatted and dehydrated avocado seed meal on performance and blood biochemistry in broiler chickens. A total of 720 one-day-old male chicks (Ross AP95) were distributed in a completely randomized design with five inclusion levels (0.0, 1.5, 3.0, 4.5, and 6.0%), eight replicates, and 18 birds per experimental unit. Diets were based on corn and soybean meal, and during the experimental period (1 to 21 days), birds had ad libitum access to feed and water. At 21 days of age, feed intake, body weight gain, and feed conversion ratio were evaluated, and one bird per experimental unit was randomly selected for blood collection from the brachial vein to determine serum concentrations of albumin, aspartate aminotransferase, cholesterol, creatinine, alkaline phosphatase, glucose, total protein, and triglycerides. Data were subjected to tests of residual normality and homogeneity of variances, followed by analysis of variance. When a significant effect was observed, polynomial regression analyses were performed. All analyses were conducted adopting a significance level of 5%. No differences ($P > 0.05$) were observed in feed intake, body weight gain and blood biochemistry at 21 days of age. However, feed conversion ratio was influenced by the inclusion levels of defatted and dehydrated avocado seed meal, presenting linear and quadratic effects ($P = 0.001$), with a better fit to the quadratic model ($R^2 = 0.94$). The optimal point estimated by the model was obtained at 2.1% inclusion of the ingredient, corresponding to a feed conversion ratio of 1.344 g/g. This result may be associated with the presence of phenolic compounds and the fibrous fraction of avocado seed, which may favor digestion and nutrient utilization in broiler chickens. It is concluded that defatted and dehydrated avocado seed meal can be included in broiler diets up to the level of 2.1%, promoting improved feed efficiency without compromising performance or the blood biochemical profile of the birds.

Paraprobiotics and metabolic stress in peak-laying hens: implications for yolk lipid stability

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Commercial laying hens have been selected for high productivity, which consequently intensified the metabolic pressure on the liver. During peak, elevated hepatic lipogenesis and yolk precursor synthesis may result in susceptibility to liver lipid accumulation and oxidative stress, thus affecting the production performance, health, and may contribute to the reduction of yolk stability. Evidence that oxidative stress due to the higher metabolic demand may impair bile acid homeostasis, hepatic stress, and progressive reproductive decline, with accelerating ovarian aging and shorter clutch in the later phase. Environmental stressors present in intensive poultry systems can further exacerbate this physiological vulnerability. Therefore, nutritional strategies that can improve hens' resilience during this critical period can improve the efficiency of production and the quality of eggs. Probiotics of Lactobacillus spp. have been widely used to promote gut health through microbiota modulation, resulting in improved performance and health of the host. However, the use of live microorganisms raises concerns regarding the potential transfer of antimicrobial resistance genes through conjugative plasmids and transposons. Paraprobiotics are defined as inactivated microbial cells or their structural components that retain biologically active molecules capable of conferring health benefits to the host (e.g., peptidoglycans, lipoteichoic acids, wall teichoic acids, and exopolysaccharides). These structures can interact with pattern recognition receptors in the intestinal epithelium, triggering intracellular signaling pathways such as nuclear factor kappa B and mitogen-activated protein kinase, thereby modulating inflammatory responses and enhancing antioxidant defense. Increasing evidence across species suggests potential effects on host lipid metabolism and oxidative balance. Overall, paraprobiotics represent a promising, safe, and stable nutritional strategy to mitigate oxidative damage and metabolic stress in modern laying hens under intensive production, with potential impact on egg quality and consequent effects on reproductive characteristics in the later laying phase.

Modulation of systemic metabolic phenotype and growth performance in broiler chickens following in ovo administration of myo-inositol

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Myo-inositol (MI) is a bioactive metabolite involved in phosphoinositide signalling, glucose metabolism, and insulin sensitivity [1] and directly modulates cellular energy sensing via AMP-activated protein kinase [2]. Given the central role of these pathways in metabolic regulation, MI may represent a candidate for metabolic programming in broiler chickens to support post-hatch development; however, this concept was not investigated in poultry. Therefore, the aim of the present study was to determine whether increasing MI availability during late embryogenesis leads to lasting modifications in growth performance, plasma metabolomic profiles, and hepatic AMPK- and insulin-related signalling pathways in broiler chickens. We hypothesized that in ovo MI administration would induce sustained alterations in systemic metabolic phenotype, accompanied by modest improvements in growth performance. A total of 480 Ross 308 broiler eggs were incubated under standard conditions, and on embryonic day 17, 440 viable eggs were allocated to 4 groups. Two MI-injected groups received intra-amniotic injections of MI (1 mL, 12 or 24 µmol MI) and two no-MI groups comprised a saline-injected positive control (1 mL, 0.9% NaCl) and a non-injected negative control. After hatch, 384 chicks were placed in 32 floor pens (12 birds per pen; 8 pens per group) and reared under identical nutritional and environmental conditions until day 35. Growth performance was monitored throughout the trial. At day 35, one bird per pen was sampled for blood and tissue collection. Plasma metabolomic profiling was conducted by Biocrates Life Sciences AG using AbsoluteIDQ™ p180 kit. Hepatic expression of insulin- and energy-related signalling proteins (INSR, p-INSR, PI3K, Akt/PKB, p-PKB, mTOR, p-mTOR, AMPK, p-AMPK) were quantified by Western blot and normalized to β-actin. MI concentrations in plasma, liver, kidney and breast muscle were quantified enzymatically, and expression of inositol monophosphatase 1 (IMPase 1) in liver and muscle and myo-inositol oxygenase (MIOX) in kidney was determined by ELISA. Statistical analyses were performed using Proc MIXED in SAS (version 9.4) with planned contrasts comparing MI-injected and no-MI groups. Plasma metabolomics revealed that MI-injected birds had higher concentrations of C2 and C9 acylcarnitines, threonine, sarcosine, and several phosphatidylcholine and sphingomyelin species. This pattern suggested altered mitochondrial substrate handling and acetyl-CoA buffering, modifications in amino acid and one-carbon metabolism, and remodelling of membrane lipid composition. Serotonin and the serotonin/tryptophan ratio were lower in MI-injected groups, indicating altered peripheral serotonergic regulation. In liver, Western blot analysis showed lower expression of p-INSR, p-AMPK and mTOR in MI-injected groups compared with no-MI ones, while INSR, PKB, p-PKB, PI3K, and p-mTOR did not differ. MI concentrations in plasma and tissues, and expression of IMPase 1 and MIOX, were unchanged. MI-injected birds showed lower average daily gain throughout rearing, with unchanged feed conversion ratio and improved survival during the first three weeks. In ovo MI administration induced persistent modulation of selected plasma metabolites and hepatic signalling proteins in broilers, supporting our hypothesis of altered systemic metabolic phenotype. Despite unchanged growth performance, improved early survival suggests functional relevance of the observed metabolic modulation and justifies further investigation. [1] Gonzalez-Uarquin et al. 2020. Poult. Sci. 99: 893-905 [2] Hsu et al. 2021. Mol. Cell. 81: 3803-3819

How Artificial Intelligence Could Transform Pig Farming in the Next Decade

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Pig farming is facing multiple and sometimes contradictory challenges: improving competitiveness while maintaining high product quality, complying with increasingly demanding regulations, and responding to growing societal expectations regarding animal welfare and antibiotic reduction. At the same time, producers must deal with strong market volatility and rising production costs. These challenges create a strong need for new technological solutions capable of supporting farmers in their daily decision-making. Artificial intelligence (AI), combined with the rapid development of Internet of Things (IoT) technologies, opens new perspectives for the next decade. Sensors, cameras, microphones and connected equipment can continuously monitor animal behaviour, feed intake, body weight, environmental conditions and health indicators. By integrating these large data streams, AI systems can transform raw information into real-time decision-support tools for farmers. Several promising applications are already emerging. Precision feeding systems could dynamically adapt feed composition or ration levels according to animal performance, feed prices or production objectives, helping to reduce feed costs and environmental impacts while improving efficiency. Computer vision technologies may allow automatic counting of animals, estimation of body weight or behavioural monitoring. Health monitoring systems based on sound analysis or activity tracking could detect early signs of disease before clinical symptoms appear. Artificial intelligence could also improve the management of livestock buildings. AI-based systems may detect anomalies in ventilation or ambient conditions and trigger alerts before problems arise. By integrating weather forecasts, intelligent control systems could anticipate sudden temperature variations and automatically adjust ventilation or heating parameters. In the context of climate change, feeding strategies and environmental management could also be adapted during heat stress periods to maintain animal performance and welfare. Beyond the farm itself, AI could support stronger integration between farms and the rest of the value chain. By connecting farm data with producer organisations, feed suppliers or slaughterhouses, digital systems could enable real-time techno-economic analysis of production. Such integration would allow farmers to anticipate feed orders, optimise stock management and adjust production strategies according to market demand or slaughterhouse specifications. More advanced systems may ultimately rely on digital twins of farms: virtual models continuously updated with real-time data that allow simulation of different management scenarios. These tools could support farmers in exploring alternative strategies. Generative AI may also simplify data recording and advisory processes. Farmers could describe their observations verbally while walking through the barn, while AI systems automatically transcribe and structure this information into farm databases. Based on data, intelligent systems could generate semi-automated action plans or management recommendations. The role of agricultural research is also evolving. Researchers are no longer only producers of knowledge but increasingly integrators of data, designers of digital infrastructures and facilitators of collaboration between farmers, technology providers and industry stakeholders. Combining animal science, engineering, data science and economics will be essential to transform technological potential into reliable and usable solutions. Over the next decade, the successful integration of AI in pig farming will therefore depend not only on technological progress but also on the capacity of research communities to co-design these systems together with farmers and industry partners.

How AI Is Reshaping the Role of the Pig Researcher

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Since the public release of ChatGPT three years ago, artificial intelligence (AI) has become increasingly integrated into pig research workflows. What began as an optional tool for early adopters has rapidly evolved into standard infrastructure for writing, analysis and scientific communication. AI-assisted drafting is now becoming more widely accepted, and many institutions require disclosure of how AI contributed to research outputs. Across the research process, AI has expanded from a writing assistant to a multipurpose tool. General chatbots and domain-specific systems support brainstorming, structuring information, learning new methods, coding tasks, documentation, data analysis and statistics. They also assist with preparing abstracts, organizing ideas, planning studies and drafting manuscript sections. These tools increasingly shape how pig researchers structure and execute their work. AI is also contributing to changes in experimental approaches. Models can process video, audio and sensor data to monitor behaviour, health and welfare. Current applications include detecting respiratory problems, estimating body weight, quantifying activity and identifying behavioural changes in pig pens. In pig research settings, AI supports automated behavioural annotation pipelines, scoring of posture and activity, and the fusion of multi-sensor signals into interpretable welfare indicators. In addition AI can support the transition from raw data to scientific output. Tools can assist in organizing multi modal data sources, extract relevant findings, highlight patterns, and create visual summaries that support communication with colleagues, stakeholders, and farmers. Tailored systems can also translate behavioral or sensor signals into accessible outputs, such as farm-specific dashboards or chatbots that provide situation-dependent advice. The increasing use of AI may require introducing methodological and ethical considerations. Hallucinations, misinterpretations of biological signals and errors in summarization pose risks for research quality. Furthermore, researchers must also be able to distinguish between general AI outputs based on public knowledge and context-specific analyses derived from private or confidential datasets. Setting requirements for online models, protected environments and local computation, depending on data sensitivity, reproducibility and the need for traceable model behaviour. Overall, AI is not replacing but reshaping the role of pig researchers. Domain expertise remains essential for formulating hypotheses, designing experiments, interpreting biological signals and ensuring ethical oversight. In data-rich production environments, the researcher increasingly acts as an integrator who combines biological knowledge with computational tools. This presentation provides participants an overview of how AI can be integrated across all stages of the research process from idea generation and experimental design to behavioural monitoring, data analysis and communication. Examples will illustrate how tailored models, sensor-based pipelines and farm-specific tools can support research and applied decision-making. The presentation will highlight current limitations, risks such as hallucinations, and opportunities for collaboration in developing accurate, context-specific AI solutions.

Contributions of physiological sensors and AI-assisted videos to the study of physiological and behavioural adaptative mechanisms in growing pigs facing a heat wave

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Heat stress (HS) is a growing concern due to global warming, especially for livestock species with limited thermoregulatory capacities such as pigs. Several physiological and behavioural mechanisms are triggered to facilitate pig's adaptation to elevated temperature, which is however generally associated to reduced growth performance. Moreover, there is a wide individual variation in how quickly and effectively pigs may adapt to heat. The aim of this study was to describe the real-time diurnal and nocturnal variations in some physiological and behavioural indicators in growing pigs facing a heatwave. Twelve crossbred (Large White × Landrace) × Piétrain) male pigs were reared in individual indoor pens, with an ambient temperature set to 22°C for 18 days (thermoneutral conditions: [TN]), and then at 32°C for the subsequent 6 days (heat stress challenge [HS]). All pigs were fitted with an indwelled venous catheter, and time-series blood samples were collected every hour at one day during TN and at one day during HS, in order to study the circadian variations of plasma glucose levels. Salivary cortisol levels were measured at two days during TN and each day of HS. Continuous monitoring of core temperature (Tcore) were obtained from sensors (Anipill, France) implanted intramuscularly. A continuous glucose monitoring (CGM) system (Dexcom G6, Dexcom Clarity, US) was inserted subcutaneously the day before HS, and rest during the following 6 days. Measurements were wirelessly transmitted to dedicated recorders. Pigs were fed ad libitum thanks to automated electronic feeders. Pens were video recorded by infrared cameras. We developed AI-based assisted classification of pig behaviours using a convolutional neural network (YOLOv11) and a minute frequency sampling rate, to extract the following behavioural traits (frequency of occurrence): visit to the drinker, head in the feeder, lying down, standing up, sitting, moving, sniffing or nosing the littermate in the neighbouring pen. Data were analysed byANOVA to assess the effects of the period (TN, HS) and circadian rhythm (nocturnal, diurnal). This reveals that salivary cortisol levels were superimposed on the circadian rhythm and, on average, were greater in HS than in TN (0.97 + 0.80 vs. 0.68 + 0.63 ng/mL, $P < 0.001$). On average, Tcore was higher under HS than under TN (39.6 + 0.8 vs. 39.1 + 0.4°C, $P< 0.001$), which indicates perturbations of thermoregulatory mechanisms. There was no difference in mean Tcore between diurnal and nocturnal periods. Daily mean plasma glucose concentrations were similar between TN and HS. The CGM data reveal a large inter-individual variability in metric for peaks and nadirs of glucose levels, in nocturnal as well as in diurnal times. Glycemic excursions and Tcore dynamics were generally synchronized in the HS period. The AI-assisted video classifications revealed behavioural shifts between HS and TN periods, with more frequent visits to drinkers, less frequent visits to feeders, more sitting and reduced social interactions ($P < 0.001$). Data from the automatic feeder confirmed that pigs under HS had a lower feed intake ($P < 0.02$) and shorter duration of meal ingestion ($P = 0.01$) than pigs under TN. In conclusion, sensor-based monitoring systems are helpful to detect (a) synchronic deviations of physiology under heat stress in growing pigs, whereas AI-assisted videos may be reliably used to detect warning signals of health and welfare deviations. This work was funded by the French National Research Agency under the project Wait4 (ANR-22-PEAE-0008).

Heat stress in weaned piglets - respiratory rate in relation to animal behaviour and temperature-humidity index

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Pigs possess limited thermoregulation mechanisms, increasing the risk of heat stress with high outdoor temperatures and limited heat dissipation in closed housing systems. Sensor systems are a promising approach for continuous monitoring and, thus, early detection of heat stress. Previously, a temperature-humidity index (THI) formula for fattening pigs was developed, which indicates an alarm situation above a heat stress threshold value of 75 (De Prekel et al. 2024). Heat stress in weaned piglets has hardly been object to research yet. It affects individual animals differently. Therefore, aim of this study was to examine animal-related parameters of heat stress in this animal group in a first step. The respiratory rate (RR) of weaned piglets (n=144) was analysed in relation to THI values and behaviour. Therefore, data were collected over a summer period of 39 days in a conventional compartment with six pens. Each pen (2.5 x 5.5 m) was equipped with climate sensors (DOL 114, dol-sensors A/S, Aarhus, Denmark) and a video camera (Axis M3206-LVE, Axis Communications AB, Lund, Sweden, top-down-view, 1,920 x 1,080px, 20 frames/s), which captured the entire pen. The mean daily THI was calculated from the assessed climate data according to De Prekel et al. (2024). Two four-day periods were selected in the data set, characterised by a mean daily THI below the threshold-value of 75 for two days before the threshold-value was exceeded for two consecutive days. Period 1 was from day 17 to day 20 of rearing, and period 2 was from day 29 to day 32 of rearing. For these two test periods, RR was visually determined in videos of a random sample of eight animals per day (n=384). Four animals were examined in the morning (8:00 a.m. to 11:59 a.m.) and four animals in the afternoon (12:00 p.m. to 3:59 p.m.). The animals were marked with coloured ear tags to distinguish them from one another. Breaths were counted for 30 seconds and breaths per minute were calculated subsequently. In addition, a half-hourly scan sampling assessed the number of animals in the manure area (MA), the number of animals lying lateral (LL), and the number of animals lying without physical contact (BC) in the entire pen. A weak positive and significant correlation between MA and RR ($\rho=0.17$; $p<0.001$) was detected, as well as between LL and RR ($\rho=0.11$; $p<0.001$). THI and RR also showed a weak positive and significant correlation ($\rho=0.12$; $p<0.001$). Between BC and RR there was a moderate positive, significant correlation ($\rho=0.30$; $p<0.001$). Respiratory rate reflected the behaviour and THI well. These first results indicate the potential of sensor-based detection of heat stress in weaned piglets. In addition to animal behaviour, the inclusion of RR as a physiological parameter is essential for the development of a climate-based early warning system. Detecting and preventing heat stress at an early stage represents a major contribution to animal welfare and health. The project was supported by funds of the Federal Ministry of Agriculture, Food and Regional Identity (BMLEH) based on a decision of the Parliament of the Federal Republic of Germany via the Federal Office for Agriculture and Food (BLE) under the strategy for digitalisation in agriculture, grant number 28DE109E18.

Effects of Temperature-Humidity Index on reproductive and productive performance in Spanish commercial sow farms

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Heat stress is a major constraint for reproductive efficiency in intensive pig systems, yet its impact under commercial conditions remains insufficiently quantified. This study evaluated the short- and medium-term impact of climate (through temperature–humidity index, THI) on key reproductive and productive indicators using long-term commercial field data combined with explainable artificial intelligence modelling. Monthly production records from 50 commercial pig farms in Spain were analysed over a 10-year period. Data were extracted from standardized herd performance monitoring systems and included services, farrowing, piglet losses and farm inventory indicators. Meteorological data, including outdoor ambient temperature and relative humidity, were obtained from weather stations located near each farm and used to compute monthly average THI values. Machine learning models (Neural Networks) were developed incorporating both contemporaneous THI and lagged THI values up to five months prior to the observed outcome. This design enabled evaluation of climatic conditions during biologically relevant stages such as insemination, early gestation and late gestation. Model interpretation was performed using SHAP (Shapley Additive Explanations) to quantify variable importance, identify critical lag periods and estimate response thresholds while accounting for farm-level effects. Delayed THI effects were consistently stronger than immediate effects. For farrowing rate, THI recorded three to four months before parturition, corresponding to the insemination and early gestation period, exerted the greatest negative impact. An effective THI threshold around 58–60 was identified, above which farrowing rate declined progressively and repeat rate increased. In contrast, piglet losses were more strongly associated with THI during late gestation, with elevated heat load preceding farrowing increasing both total mortality and early neonatal losses. Farm-specific embeddings revealed substantial heterogeneity in climatic sensitivity, indicating that management, housing design or genetic background modulate heat stress resilience. These findings demonstrate biologically coherent delayed effects of heat stress on reproductive performance and piglet survival under commercial conditions. The integration of long-term field data with explainable artificial intelligence provides a robust framework to identify critical vulnerability windows and support targeted climate-adaptation strategies in pig production systems. Results highlight the importance of incorporating temporal climate dynamics into reproductive management decisions at both farm and system levels across diverse production contexts nationally.

Development of a continuous MIR-based nutritional stress score using an experimental challenge in dairy goats

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The aim of this study was to use mid-infrared (MIR) spectra to detect nutritional stress and to construct a continuous nutritional stress score. First, we assessed whether milk spectral profiles were affected by an experimentally induced acute nutritional stress. For this purpose, MIR milk spectra were collected before and after a 2-day nutritional challenge consisting of a straw-only diet. The study involved two INRAE experimental farms (Bourges and Paris) and 377 goats. At Bourges (n = 241), challenges were conducted in March (early lactation) and June (mid lactation). At Paris (n = 136), challenges were conducted in March (early lactation) and September (late lactation). The first objective was to classify milk samples as pre- or post-challenge by training the model on one farm and predicting on the other farm. With external farm validation, the elastic net model based on spectra achieved a 1% classification error rate, whereas a binomial model based on milk yield, fat content, and protein content resulted in a 13% error rate. Then, to construct a continuous score, each MIR wavenumber was adjusted for days in milk using a linear model with 5-day DIM classes fitted on routine records from the Bourges farm (15 to 300 DIM, 11 568 MIR records). The estimated DIM effect was used to correct the challenge spectra before training an elastic net model to discriminate between pre-challenge and post-challenge samples. This elastic net model was applied to routine MIR of milk recording data from the Bourges farm (DIM-adjusted) between 15 and 300 days in milk to estimate the probability of being classified as equivalent to day 2 (post-challenge), therefore indicating a state of spontaneous nutritional stress. The data comprised 11 568 MIR spectra from 883 goats over 7 years. The predicted probability was defined as the STRESS_SCORE, ranging from 0 to 1. Among 11 568 MIR routine records, 80 samples had a STRESS_SCORE greater than 0.5. To assess biological coherence, correlations were computed between STRESS_SCORE and predicted contents of milk metabolites and fatty acids using routine MIR of milk recording data from the Bourges farm. The score showed a positive correlation with milk C18:1 cis-9 (r = 0.42 overall; range: 0.33 to 0.57 across lactation stages) and a negative correlation with de novo fatty acid content (r = −0.33 overall; range: −0.30 to −0.57). These associations suggest that this STRESS_SCORE reflects lipid mobilization. After transformation to achieve normality for STRESS_SCORE, genetic parameters were estimated using a test-day approach with a repeatability animal linear mixed model. Preliminary genetic parameter estimation showed a repeatability of 0.34 and a heritability of 0.22 (± 0.03). The STRESS_SCORE derived from MIR spectra is an indicator of nutritional stress and reflects lipid mobilization. It shows moderate repeatability and heritability, and supports the use of controlled experimental challenges to develop phenotyping indicators.

Physiological validation of heart rate variability as a sensor-based indicator of environmental and metabolic disturbances in grazing dairy cows

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Precision Livestock Farming through sensor-derived physiological signals could detect metabolic and environmental disturbances at early stages. Heart rate variability (HRV), measured using wearable sensors, reflects autonomic nervous system activity and has been proposed as a noninvasive indicator of stress and physiological imbalance in livestock. However, the physiological validation of HRV signals against metabolic biomarkers under grazing conditions remains limited. We hypothesized that HRV-derived autonomic signals could be useful as a physiological marker associated with metabolic status and environmental stress in grazing dairy cows. This study aimed to evaluate whether heart rate variability (HRV) responses are associated with physiological markers of metabolic status and environmental stress, and to assess their potential use as sensor-based indicators for early detection of disturbances in grazing dairy cows. A cross-sectional study was conducted in clinically healthy lactating Holstein cows (n = 330) under grazing conditions in the high-Andean tropics of Colombia. Arterio-venous coccygeal blood samples were collected for on-site blood-gas analysis. Subclinical metabolic alkalosis (MA) was defined as blood pH > 7.45, $HCO_3 \geq 28$ mmol/L, and/or base excess ≥ +3 mmol/L. Blood lactate concentrations were categorized as < 2.0, 2.0–4.0, and > 4.0 mmol/L. Tissue hypoperfusion was evaluated using lactate, acid–base parameters, anion gap, hematocrit, and renal perfusion indicators. Environmental conditions were continuously recorded using an on-farm meteorological station, and environmental heat load was characterized using the Temperature–Humidity Index (THI) and the Temperature–Humidity–Wind Index (THVI).. Autonomic responses were assessed through HRV monitoring using wearable heart-rate sensors (Polar system) and analyzed with Kubios software. Subclinical MA was identified in 29.9% of cows. Alkalotic cows showed higher bicarbonate and base excess and lower plasma chloride concentrations ($p < 0.05$). Blood lactate concentrations were generally low to moderately elevated and were not associated with acid–base alterations or indicators of tissue hypoperfusion. Moderate hyperlactatemia was accompanied by reduced HRV indices and increased LF/HF ratio, consistent with sympathetic activation and higher environmental heat indices. These results support the hypothesis that HRV-derived autonomic responses reflect physiological adaptations to environmental stress rather than metabolic hypoperfusion. The findings provide physiological support for the use of HRV-based wearable sensors as indicators within precision livestock farming systems, contributing to early-warning monitoring of environmental and metabolic disturbances in pasture-based dairy production.

Actionable indicators derived from milk fatty acids to assess rumen health, negative energy balance and production losses in dairy cows

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Metabolic health is essential for optimal production performance, fertility, and longevity in dairy cows. Early detection of excessive fat mobilization and impaired rumen function enables timely interventions at both group and individual cow level, helping to prevent milk losses and improve fertility. However, existing monitoring tools often lack sensitivity and provide limited actionable guidance for farmers and advisors. Milk fatty acids (MFA) reflect metabolic status but vary with lactation stage, parity, breed, and season, making them non-actionable when interpreted in isolation.The objective of this study was to develop actionable risk indicators based on the MFA profile while accounting for these covariates. Milk fatty acid profiles, predicted from mid-infrared spectra, were obtained from 181,794 milk samples collected through the national milk recording (MR) program at 52 farms over a four-year period (2021–2024). The dataset comprised 14,261 unique lactations and was combined with auxiliary cow data, daily milk yield records, and herd-level MR information on fat and protein yield.Three MFA-based risk metrics were developed to predict poor rumen function (days in milk [DIM] 0–450), negative energy balance (DIM 0–100), and early-lactation milk losses (DIM 0–42), with production losses evaluated over the first 150 days of lactation. Each metric classified cows into low, medium, or high risk categories. Odds ratios were calculated to quantify differences in milk losses between risk classes, and MFA-based risks were compared with existing ketosis alerts based on ketone measurements. A second validation study, conducted on 27 farms in 2025–2026, evaluated milk losses and farmer experiences.Cows classified as high risk based on MFA profiles were up to three times more likely to fall within the worst 20% quantile for milk losses compared with low-risk cows. Conversely, cows with a low-risk MFA profile were up to 3.5 times more likely to be in the lowest 20% for milk losses than high-risk cows. The MFA-based risk metrics demonstrated higher sensitivity than current ketosis alerts. In the second validation study, cows identified as high risk for both negative energy balance and rumen dysfunction exhibited an average milk loss of 0.6 kg/day, corresponding to 74% greater losses than cows with low risk scores. These MFA-based risk indicators enable farmers to optimize rations at group level and to identify individual cows requiring additional attention, thereby helping to prevent milk losses and improve metabolic management.

Quantification of drinking-induced rumen temperature change in dairy cows

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Intraruminal bolus temperature is widely used for health and physiological monitoring in precision dairy systems; however, the magnitude and persistence of drinking-induced thermal changes remain insufficiently quantified. We hypothesized that drinking behavior induces a distinct and temporally persistent rumen cooling signature that remains detectable at a 10-minute temporal resolution. We quantified the drinking-associated rumen temperature change using synchronized multimodal sensor measurements. We monitored seven late-gestation dairy cows with access to two drinkers (A and B) over a 4-month experimental window (range of days among cows: 6-10). We identified drinking behavior using manually video-labelled single-cow events within each interval (216 10-minute intervals, 3.05% of observations). We classified an interval as drinking if at least one drinking bout occurred within the 10-minute window. Rumen temperature was recorded using an intraruminal bolus (SmaXtec Classic Bolus, SmaXtec, Graz), providing both raw temperature and manufacturer-corrected "temperature without drink cycles". We recorded external water temperature from two trough loggers (HOBO Pendant MX2201 Temperature Sensor, LI-COR, Lincoln). Baseline temperature was defined as each cow's mean rumen temperature during intervals without video-confirmed drinking, excluding the drinking interval and the subsequent two intervals. Raw rumen temperature during video-confirmed drinking intervals averaged 37.87°C compared to 39.15°C during non-drinking intervals, corresponding to a mean cooling magnitude of −1.29°C (Cohen's d = 1.25; interval-level pooled SD). Across cows, the mean within-animal cooling effect was −1.10 ± 1.15°C (SD across cows). When accounting for temporal dynamics, peak cooling within the subsequent 20 minutes reached −4.14 ± 1.34°C relative to cow-specific non-drinking baseline, with nadir typically occurring in the first interval following drinking and observed in all seven cows (range −2.57 to −6.25°C). These findings demonstrate a substantial rumen temperature decrease that remains clearly detectable despite 10-minute aggregation. Analysis of post-drinking intervals revealed a sustained cooling trajectory. The difference between raw and corrected temperature averaged +1.56°C in the final drinking interval and peaked at +3.55°C in the first 10-minute interval following drinking, remaining elevated for up to 30 minutes before gradually returning toward baseline. Manufacturer-corrected temperature showed no appreciable difference between video-confirmed drinking and non-drinking intervals (Δ = +0.01°C). The correction magnitude averaged +1.56°C during drinking intervals and +0.27°C during intervals without video-confirmed drinking, indicating that the algorithm substantially attenuates drinking-associated cooling while applying minor adjustments outside labelled events. During video-confirmed drinking intervals, trough water temperature averaged 5.74 ± 1.83°C (Drinker A) and 6.72 ± 1.75°C (Drinker B). Collectively, these results demonstrate that drinking behavior induces an immediate cooling signature of approximately 1.3°C within drinking intervals and a peak cooling magnitude of approximately 4°C within 20 minutes. This signal can bias interpretation of raw bolus temperature when the objective is physiological monitoring, but it also provides a robust behavioral marker for drinking activity, underscoring the necessity of behavior-aware pre-processing in precision dairy monitoring.

FRESHEEP&GOAT: Adapting dairy sheep and goat barns to reduce heat stress due to climate change

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Dairy sheep and goats are common livestock activities on both sides of the Pyrenean territories where farmers share pastoral traditions and produce renowned cheeses which are socially, economically and culturally important for their communities. With this aim, as a part of the EU's initiative INTERREG-POCTEFA, a cooperation project was launched in 2026 to support regional development and to address shared challenges related with climate change in French and Spanish dairy sheep and dairy goat farms in the Pyrenean cross-border territories. Climate change is a significant concern which affects ruminant farming due to the rise of temperatures and the increased frequency and intensity of heat waves. Heat stress impacts on animals' health and well-being and impairs the productivity and profitability of dairy farms, as well as the quality of their products. Particularly exposed to climatic hazards, the POCTEFA territories are a key area for the development and implementation of appropriate practices and equipment. The FRESHEEP&GOAT EFA175/06 project aims to strengthen the resilience of dairy sheep and goat farms by adapting the farm buildings to different strategies to limit the effects of heat stress. It relies on the use common methodology and on collaboration between scientific, technical, and professional stakeholders to identify innovative and effective and cost-benefit solutions. The production, design, and environmental parameters of the buildings on 30 sheep and goat farms, including experimental and commercial farms, will be monitored on both sides of the French-Spanish border through 2026 to 2028. An algorithm linking meteorological data with environmental conditions and building design will also be developed. All these measures will make possible to establish solid benchmarks and ensure their transferability to commercial farms and its reproducibility on a larger scale.

Between-cow variation of responses to a subclinical ketosis challenge in early-lactating dairy cows

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Subclinical ketosis (SCK) in dairy cows shows marked inter-individual variability with some cows developing high beta-hydoxybutyrate (BHB) levels during negative energy balance (EB), while others do not. The mechanisms underlying this variability are not fully understood. The present pilot study investigated between-cow variation and potential contributing factors in early-lactating cows challenged by an experimental SCK-inducing feeding model (energy oversupply ante partum and energy undersupply post partum; n=8) and control cows (CON) fed according to requirements (n=8). Daily data (1- 28 d post partum) of serum BHB, non-esterified fatty acids (NEFA), glucose, aspartate aminotransferase (ASAT), alanine aminotransferase (ALAT), and gamma-glutamyl transferase (GGT), urine acetoacetate, milk acetone, dry matter intake, milk production, and body condition score (BCS) were collected, and energy intake and EB were calculated. Principal component analysis (PCA) and hierarchical cluster analysis were performed in R to explore whether cows within treatment groups formed distinct subgroups, and if so, to identify the characteristics that differentiated these groups. As intended by the experimental feed model, the EB of SCK cows was lower (mean: –32.4 MJ NEL/d) than that of CON cows (mean: –8.99 MJ NEL/d). On average, cows in the SCK treatment had serum BHB concentrations > 1.2 mmol/L (the diagnostic threshold for SCK) for 10 d (range of absolute values: 0.36 – 4.76 mmol/L; mean: 1.21 mmol/L), compared to 2 d in CON cows (range of absolute values: 0.43 – 2.27 mmol/L; mean: 0.85 mmol/L). Within both SCK and CON cows, cluster analysis identified two distinct groups of cows. For both treatments, the cluster assignment aligned with the directions of the first PCA axis, with cluster 1 cows on the positive side and cluster 2 cows on the negative side of the axis. For SCK cows, cluster 1 cows were associated with greater dry matter intake and milk yield and peaks of BHB, NEFA, ASAT, milk acetone and urine acetoacetate; cluster 2 cows with higher EB, BCS, and peaks of glucose, NEFA and GGT. For CON cows, cluster 1 cows were associated with greater energy and dry matter intake and EB, parity number, body weight and glucose and ALAT peaks; cluster 2 cows with higher milk acetone and urine acetoacetate peaks. In both treatment groups, distinct subgroups were present, reflecting high between-cow variation. In CON cows, clusters were mainly driven by intake capacity and energy intake, whereas in SCK cows, clusters were further influenced by metabolic markers of energy status and liver function. These findings indicate that cows vary in their energy and metabolic traits contributing to subgroup differentiation in both SCK-challenged and control cows. Ongoing omics analyses may offer insights into the underlying physiological mechanisms.

Temporal dynamics of breath acetone and blood β-hydroxybutyrate in early lactation dairy cows

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In early lactation, Holstein cows frequently experience negative energy balance and increased ketogenesis, raising the risk of subclinical ketosis (SCK). Diagnosis of SCK is commonly based on β-hydroxybutyrate (BHB) concentrations (≥ 1.2 mmol/L) measured from blood samples, requiring invasive collection. In contrast, breath acetone (BA) can be assessed non-invasively, making it a promising alternative diagnostic approach. A non-randomized controlled study was designed to compare longitudinal profiles of BA and BHB and evaluate BA as a non-invasive SCK indicator. Thirty-one healthy multiparous Holstein cows were allocated 42 d antepartum (ap) to one of two treatments based on body condition score (BCS). Cows with BCS ≥ 2.75 (n = 20) received SCK-inducing diets (high-energy diet ap, low-energy diet until 35 d postpartum (pp)), whereas cows with BCS < 2.75 (n = 11) were fed a standard diet (CON) according to requirements . Daily measures included dry matter (DM) intake (ap + pp), milk yield (pp), blood BHB (pp) and BA (pp). In addition, BHB and BA were assessed weekly during ap. Statistical analyses included linear mixed-effects models (fixed effects: treatment, day pp, interaction, baseline ap; random effect: animal), Spearman's rank correlation and principal component analysis (PCA). To evaluate time lags between BA and BHB, datasets were shifted by −3 to +3 days and analyzed using linear mixed-effects models, with animal as a random intercept. BA effects were further decomposed into within- and between- cow components. Receiver operating characteristic (ROC) analysis was performed, and area under the curve (AUC) and Youden's J were calculated to determine the optimal BA cut-off. During ap, cows in the SCK group had higher DM intake than CON cows ($p < 0.001$). Postpartum, SCK cows showed lower DM intake but higher milk yield than CON cows (both $p < 0.001$), resulting in a more pronounced negative energy balance in the SCK group ($p < 0.001$). BA and BHB were moderately correlated in the SCK group ($r = 0.63$, $p < 0.001$) and weakly correlated in the CON group ($r = 0.28$, $p < 0.001$), with a strong association observed at BHB concentrations > 2.6 mmol/L ($r = 0.74$, $p < 0.001$). Both BA and BHB showed substantial inter-individual variability, as reflected by large coefficients of variation (BHB: 44.7–47.1%; BA: 56.4–90.4%). PCA demonstrated separation between SCK and CON, with PC1 explaining 37.4% and PC2 explaining 10.9% of the total variance. BA was positively associated with blood BHB across all time shifts, strongest at d 0 ($\beta = 0.19$ mmol/L BHB per 1 ppm BA, $p < 0.001$). When BA was decomposed into within-animal and between-animal components, both increases in BA within animals over time ($\beta = 0.18$) and higher mean BA concentrations between animals ($\beta = 0.31$) were associated with higher BHB concentrations ($p < 0.001$). ROC analysis showed good discrimination of BA for detecting cows with BHB ≥ 1.2 mmol/L (cut-off 1.2 ppm; sensitivity 0.81, specificity 0.71, AUC 0.84). The association and temporal alignment of BA with BHB support BA as a biomarker for SCK. However, marked inter-individual variability in longitudinal ketone dynamics emphasizes the importance of analyses at the individual animal level.

Variability of urinary traits in lactating Holstein cows and their associations with milk quality traits

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Monitoring urine composition is a valuable option for assessing animal health and physiological status, including the detection of nutritional imbalances and renal disorders. Although 24-h total urine collection is considered the gold standard, its application under commercial farming conditions is unfeasible due to housing constraints, collection procedures, and animal stress. Spot urine sampling offers a practical alternative; however, adjustment for urine concentration—through specific gravity—is required to account for individual variability. Building on a large-scale sample set, this study aimed to (i) evaluate the effects of stage of lactation and parity on urinary traits and (ii) investigate associations between urinary and milk traits to demonstrate which urine traits provide independent information. Spot urine and milk samples were collected from 1,271 Holstein cows across 17 farms in northern Italy. Farms were visited repeatedly, with different cows randomly sampled at each visit. Urinary pH, specific gravity, urea, protein, creatinine, allantoin, uric acid, glucose, and the protein-to-creatinine ratio were determined using reference methods. Milk composition was analysed using infrared spectroscopy and flow cytometry. Generalized linear mixed models were applied to assess sources of variation in urinary traits, including urinary specific gravity (as a covariate), stage of lactation and parity as fixed effects, and herd-test-date as a random effect. Pearson correlations were calculated within urinary traits and between urinary and milk traits. Urinary traits showed wide variability, with the protein-to-creatinine ratio exhibiting the greatest variability, whereas pH and specific gravity were the least variable. Specific gravity was significantly affected by stage of lactation and parity and explained a substantial proportion of the variability in all urinary traits, confirming its strength in adjusting for urine concentration. Stage of lactation influenced all specific-gravity adjusted urinary traits except pH and uric acid, whereas parity significantly affected only creatinine concentration. Most nitrogenous compounds were most concentrated in urine in early lactation and followed distinct temporal patterns across lactation. Herd-test-date accounted for a considerable proportion of variance, particularly for urinary pH and urea concentration. Strong correlations were observed within urinary nitrogenous compounds, whereas associations between urinary and milk traits were generally weak, confirming their independence in diagnostic value. Only milk urea concentration was closely correlated with urinary total nitrogen and urea concentrations. Milk urea concentration showed the strongest positive correlations with urinary total nitrogen and urea. These findings provide a robust baseline for interpreting urinary traits in lactating cows and identify key sources of physiological variability, thereby laying the foundations for the use of spot urine samples in diagnostic and herd management applications. This study received support from the project Cool-Cow 2023 of the Breeders Association of Veneto Region (ARAV, Vicenza, Italy).

Impact of the Bulmer effect is stronger than typically assumed when selecting on polygenic traits

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Genetic variance is essential for genetic improvement through either natural or artificial selection. As a result of selection, the genetic variance decreases over time. A large part of this reduction is the result of a transient phenomenon known as the "Bulmer effect", which originates from the creation of negative covariances between loci. Usually, the infinitesimal model is assumed to account for the Bulmer effect when estimating breeding values and genetic variance components. However, there is increasing evidence that breeding goal traits are affected by very many loci that do not segregate independently because the number of chromosomes is limited. This creates linkage between loci, which influences the extent of the Bulmer effect. Here, we investigate the impact of linkage on the reduction in genetic variance under selection. Bulmer has shown that the reduction in genetic variance is a function of the harmonic mean of the recombination rate (rH) between loci. The rH is 0.5 when all loci are on separate chromosomes, resulting in a drop of for example ~21% of the genetic variance for mass selection on a trait with a heritability of 0.4 and a selection intensity of 20%. With 30 chromosomes of 1 Morgan each, the rH is ~0.33 when 30,000 loci are randomly spread along the genome, resulting in a drop of for example ~28%. We confirmed those predictions by simulating a population under either mass or genomic selection for 50 generations for a single trait with a heritability of 0.4 affected by 2000 additive loci, followed by 500 additional generations of random selection. The loci were either unlinked, by placing each locus on a separate chromosome, or in linkage, by placing the loci randomly over 10 chromosomes of 1 Morgan each. After 50 generations of mass selection, the loss in genetic variance due to the Bulmer effect was ~17% when loci were unlinked and ~34% when loci were linked. After selection was stopped, the genetic variance substantially increased in the first generations, mainly due to the breakdown of negative covariances between chromosomes. When loci were unlinked, the complete genetic variance was restored within seven generations. However, with linked loci, about 150 to 250 generations of random selection were needed before all negative covariances were broken down and the genetic variance was completely restored. With genomic selection, the Bulmer effect was slightly larger than with mass selection, both when loci were unlinked and linked. Altogether, our results show that the reduction in genetic variance due to the Bulmer effect can be considerably larger than typically predicted from the infinitesimal model, and that it takes much longer for the genetic variance to return to its original value. Therefore, the use of infinitesimal model assumptions, as for example done in the Animal Model, can create bias when estimating breeding values and genetic variance components.

Loss of Genetic Variance in Simulation Studies: Comparing the Effect of Selection on Classical and Deep Learning based Genetic Simulation Models

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Stochastic simulation of breeding schemes provides a time- and cost-effective means of studying the effects of selection decisions in breeding programs. However, in several simulation studies, the long-term consequences of selection have been observed to lead to rapid exhaustion of genetic variance. These outcomes differ from real-life observations that we aim to replicate, thereby highlighting a fundamental limitation of current classical quantitative genetic simulation models. Deep learning (DL) models have demonstrated promising results in capturing complex interactions essential for maintaining genetic variance; thus, we hypothesize that DL-based genetic simulation models may preserve more genetic variance than classical models, because the biological pathways underlying complex traits exhibit interactions that classical models ignore. The objectives of this study were to compare classical and DL-based genetic simulation models with respect to retained genetic variance. Topigs Norsvin provided the genotype and phenotype dataset comprising 13,944 dam-line boars of different generations, which served as the founder population for the full-sib pig breeding program simulated using ADAM-Multi. The founders' genomes comprise 18 autosomal chromosomes and 35,977 biallelic SNPs, of which 12,000 were identified as QTLs. Haplotypes from founders were randomly sampled to generate unique genotypes for individuals before selecting the base population. Phenotypes were simulated using two classical quantitative genetics models: one with additive, dominance effects, and additive-by-additive epistatic effects (ADAA), and a model that includes all pairwise epistatic interactions (ADAAAAADDD). Simulating phenotypes for DL-based models (DL_medium and DL_complex) required training models using the founders' biallelic QTLs and phenotypes. The trained QTL weights were saved and served as a complex genotype-to-phenotype map. These weights were multiplied by the offspring's QTL genotypes to generate the total genetic value (TGV), which served as the phenotype. The parents (20 males and 200 females) of the next generation were selected based on TGV and mated randomly at a 1:10 male-to-female ratio. The simulation process was repeated for 40 generations and replicated 10 times. Our results showed a 202% increase in the initial additive genetic variance of the DL_complex model, with retention of 88% of its total genetic variance, while the DL_medium model retained 35% and 26% of its initial additive and total genetic variance, respectively. In contrast, the classical models retained 38-39% of additive and 40-48% of total genetic variance. In conclusion, a complex DL-based genetic simulation model exhibited greater retention of genetic variance. This might be due to the DL model's architectural properties, which intrinsically capture complex interactions that are converted to additive variance by selection; hence, affirming the role of non-additive genetic effects in maintaining long-term genetic variation.

Impact of non-additive genetic architecture, QTL number, and reference population size on the relative performance of machine learning and GBLUP models

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Using machine learning (ML) models for genomic prediction has generated widespread interest in animal and plant breeding. The benefit of ML models over GBLUP likely depends on the ML model used, the size of the reference population, and the genetic architecture of the trait. Because ML models may capture complex interactions, it is of interest whether ML models show benefits over GBLUP with non-additive component (GBLUP-ADE) in the presence of non-additive genetic effects. The objective of this study was to compare the accuracy of different ML models with that of GBLUP-ADE under different non-additive genetic architectures, QTL numbers, and reference population sizes for prediction of total genetic values (TGV) and breeding values (BV). We used three representative ML models, random forest (RF), support vector regression (SVR), and multilayer perceptron with residual network (MLP-ResNet). Our results showed that the benefit of ML models over GBLUP-ADE depended primarily on the number of QTL underlying the trait, and to a lesser extent on the reference population size and genetic architecture. For TGV prediction, RF showed a benefit over GBLUP-ADE only when the number of QTL was 50 under a high level of dominance and a small reference population size. MLP-ResNet showed benefits over GBLUP-ADE when the number of QTL was small and the reference population size was large in the presence of epistasis. In all other scenarios, ML models showed lower prediction accuracy than GBLUP-ADE. For BV prediction, only MLP-ResNet showed a slight benefit over GBLUP-ADE, and only in scenarios with a small number of QTL and a large reference population size. Our results demonstrated that ML models showed benefits in only a few scenarios, and that these benefits were small and depended on the reference population size and the underlying genetic architecture. This study also suggests that ensemble methods (RF) and deep learning models (MLP-ResNet) can capture non-additive genetic effects in genomic prediction. These findings indicate that, for selection decisions aimed at predicting BV, GBLUP remains a robust and reliable method even when non-additive genetic effects are present. Overall, our results suggest that ML models may be considered alternatives to GBLUP(-ADE) for traits for which the infinitesimal model assumption does not hold.

A novel Monte Carlo method for approximating the null hypothesis in genomic-window genome-wide association analyses

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Genome-wide association studies (GWAS) are widely used to identify genomic regions associated with quantitative traits and diseases. To overcome the limited information from individual SNPs, window-based GWAS have been proposed. However, the proportion of variance explained by genomic windows is strongly influenced by factors unrelated to causal genetic effects, including SNP density, linkage disequilibrium patterns, allele frequency distributions, population structure, and gene–environment correlations. These factors may lead to biased identification of genomic regions and spurious associations. In this study, we propose a Monte Carlo procedure to approximate the null distribution of genomic-window statistics while preserving the genomic and genealogical structure of the population. The method was applied to cold carcass weight records from the Rubia Gallega beef cattle population. The dataset comprised 99,227 phenotypic records and a pedigree of 150,904 animals, including a total of 5,627 genotyped individuals. Variance components for linear mixed model were estimated using AIREML implemented in the BLUPF90. SNP effects were obtained using the SNP-BLUP transformation, and the proportion of additive genetic variance explained by 1-Mb genomic windows was calculated using postGSF90. To approximate the null hypothesis of no association, 1,000 simulated populations were generated. Phenotypic records were simulated by sampling environmental, additive genetic, and residual effects from multivariate Gaussian distributions parameterized by the REML variance estimates. Additive genetic effects were drawn from the stationary distribution defined by the H relationship matrix, thereby incorporating both pedigree and genomic structure. For each simulated dataset the full model was re-fitted and the proportion of variance explained by each genomic window was recomputed. The distribution of simulated estimates was then used to parameterize a gamma distribution, from which empirical p-values were derived by comparing observed and simulated statistics. The conventional window-based analysis identified several genomic regions explaining more than 0.5% of the additive genetic variance, but these signals were highly heterogeneous and strongly associated with marker density across the genome. The Monte Carlo procedure revealed that the expected variance explained by genomic windows under the null hypothesis varied substantially among regions due to genomic architecture. When empirical p-values were computed from the simulated distributions, strong signals were detected in genomic regions harbouring the well-known MSTN and LCORL genes. In addition, the method identified an additional region on BTA19 with a p-value below 0.0001 and several other regions with moderate significance that were not highlighted by conventional analyses. The proposed approach generates a null distribution that accounts for genomic architecture, pedigree structure, and environmental effects included in the model. The procedure also captures potential gene-environment correlations arising from the data structure. Consequently, the comparison between observed and simulated statistics reduces the occurrence of spurious associations while increasing sensitivity to detect genuine signals. In conclusion, the Monte Carlo framework provides a robust strategy for evaluating genomic-window GWAS results by explicitly modelling the stochastic behaviour expected under the null hypothesis. Although computationally demanding, this approach improves the reliability of genomic region detection and may enhance downstream applications such as weighted genomic prediction or functional interpretation of GWAS signals.

Optimising GCTA for improved parallelisation of large-scale association testing

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Genome-wide association studies (GWAS) are a ubiquitous approach to identifying loci which contribute to complex traits. Livestock populations commonly have both large sample sizes, consisting of tens or hundreds of thousands of array genotyped animals, and extensive phenotypic records on numerous dairy- or beef-related traits. As such, livestock GWAS can be computationally burdensome and often require compromises subject to constraints like dividing samples into multiple independent subpopulations followed by meta-GWAS. The 1000 Bull Genomes (1000BG) consortium previously modified the GCTA (Genome-wide Complex Trait Analysis) software to consider genotype dosage and phenotype weights. Here, we further extend this version, focusing on two distinct aspects: improving computational throughput and parallelisation strategy, as well as enabling association testing with additional modes of inheritance. GCTA primarily functions as a monolithic program, with commands to calculate genomic relationship matrices (GRM), principal components (PCs), restricted maximum likelihood (REML), mixed linear model associations (MLMA) etc. However, these functions can have significantly different computational resource requirements or may be unnecessarily recalculated internally when parallelising across subsets of variants. By separating independent calculation steps and storing a binary representation of state, we implemented a more granular approach to parallelising with a REML-only step (memory-bound) and a MLMA-only (thread-bound) step. These steps also use different components of the input data, and so we implemented deferred loading of data until files were needed. Given the typical size of input files (i.e. nearly 1.5 TB of genotype files for 90k samples and 29M variants), this also reduces time wasted on input/output operations across sharded runs. GCTA also includes the ability to restrict analyses to specified genomic regions, but performs an inefficient linear scan across all variant sites. For genotypes provided in BCF/VCF format, we can directly leverage the indexed files to only extract the specified genomic regions rather than passing over the entire file, again reducing unnecessary input/output pressure. GCTA also mainly tests for additive associations, where genotypes are monotonically recoded under the additive inheritance model (aa: 0, aA: 1, AA: 2). Although additive associations are the most common form, other inheritance models (e.g., overdominant) and other biometrical models (where recoding accounts for allele frequency) are increasingly being used to reveal additional trait-associated loci. While the 1000BG version of GCTA could account for nonadditive recoding via the MACH dosage format, this introduced complications of “wide”- versus “long”-form data leading to high memory demands during conversion. Here, we added new functionality to allow greater flexibility of on-the-fly recoding genotypes according to user-specific models. We validated these changes by recovering recently reported non-additive loci for stature in Brown Swiss cattle. While we did not modify the core algorithms of GCTA, our reimplementation of several functions resulted in an order of magnitude higher throughput by separating memory-bound and thread-bound steps and minimizing redundant calculations across sharded runs. In addition, several quality-of-life improvements and the ability to recode genotypes under different nonadditive models also expands the range of association testing. Our version is freely available under the GPLv3 license of the original GCTA as a fork on GitHub.

Geometrical Linkage Disequilibrium between SNPs Dominance Effects and a Quantitative Trait

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Linkage disequilibrium (LD) is the non-random association of alleles at two or more loci, not necessarily on the same chromosome. LD is central to our understanding of the genetic architecture of diploid organisms. The great majority of research investigating LD between Single Nucleotide Polymorphism (SNP) genotypes and quantitative traits considers only SNP additive effects. Geometrical Linkage Disequilibrium (GLD) is defined as the angle between SNP vectors of genotypic values centered at their mean. GLD is similar to a standard LD measures such as r. Orthogonal vectors (GLD = 90°) indicate no LD, whereas collinear vectors (GLD = 0°) indicate complete LD. The objective of this study is to extend GLD to incorporate dominance effects affecting a quantitative trait. The elements of the vector of dominance effects are $(1 - f)\delta$ for heterozygous individuals and $-f\,\delta$ for both types of homozygous individuals, where δ is the dominance effect and f is the frequency of the heterozygous genotype. We report the algebraic expression for the expected value of GLD for dominance effects as a function of α (additive effect), δ, and genotype frequencies. A computer simulation was carried out using an SNP allele frequency of 0.5, a population size of 300, and 10,000 replicates per simulation set. The quantitative performance was simulated using normal deviates with a phenotypic stardard deviation (psd) of 12.44. There was a very good agreement between simulated and observed estimates of GLD. Thus, GLD for $\alpha = 1$ psd and $\delta = 1$ psd was 69.21° and 69.30° for predicted and observed dominance effects, respectively. The GLD for $\alpha = 0$ and $\delta = 1$ psd was 65.85° and 65.91° for predicted and observed dominance effects, respectively. The standard deviation among replicates was 3.32° and 3.15° for the two scenarios considered. The main advantage of GLD is computational since it only involves the dot products of vectors. Therefore, the large-scale use of GLD may facilitate detection of dominance in large datasets, such as those generated by Next Generation Sequencing.

Misunderstood matrix: How changing the incidence matrix of social genetic effects changes the trait definition

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Models including social genetic effects are a powerful tool to estimate the total heritable variation by including both direct (DGE) and social genetic effects (SGE) in the model. In these models, the SGE are linked to the phenotypes by using an incidence matrix, which is usually based on the group composition in animal breeding. In forest breeding, also the distance between the trees has been used to link the phenotypes and associated individuals. Recently, in animal breeding there is a trend of including the number of interactions between pairs of animals into the incidence matrix of SGE (ZSGE) to improve the estimation of variance components. While the use of distance in tree breeding uses information from the experimental design to scale the ZSGE, this method uses information based on observations to scale the ZSGE. Here, we hypothesize that by scaling the ZSGE based on observations instead of group composition the meaning of the estimated effects and thereby the targeted underlying traits change. Therefore, our objective was to understand how the choice of incidence matrix affects the meaning of the SGE and the resulting genetic change. We used pigs as an example and simulated a harmful behaviour with four underlying traits: (1) social tendency (= tendency to meet other pen mates), (2) tendency to bite given that two pen mates meet, (3) tendency to be bitten given that two pen mates meet, and (4) damage inflicted by the biter per biting event. Based on these four underlying traits we simulated movement and biting interactions between the pigs. Using the damage recorded on the victims as the phenotype we simulated ten generations of selection based on either SGE or total breeding values (also including DGE). We investigated three definitions of ZSGE. The three ZSGE were based on (1) pen composition, (2) scaled by number of meets and (3) scaled by number of biting events between pairs of animals. We chose the scenarios based on number of meets and number of events as this information will soon be available to breeders with the help of tracking and behaviour detection algorithms. Preliminary results show that the choice of ZSGE changed the underlying trait(s) of the estimated SGE. This resulted in different genetic gains in the four underlying traits depending on the choice of ZSGE, even though the phenotype remained damage of the victims in all cases. The SGE estimated with a ZSGE based on the number of events only targeted damage performed per biting event. In contrast, the SGE estimated with a ZSGE based on pen composition or number of meets targeted both the tendency to bite and the damage caused per biting event. Furthermore, when the selection was based on the total breeding value, the tendency of being bitten could also be reduced using a ZSGE based on pen composition or number of meets. However, it was not possible to select on direct effects when using the ZSGE based on number of events, as scaling the ZSGE by number of events resulted in no direct genetic variance in our simulation set up. In summary, scaling the incidence matrix of SGE based on behavioural observations lead to changes in the traits underlying both direct and social genetic effects. Therefore, researchers should be careful when applying such methods as it can lead to unexpected genetic change.

Deriving Metafounders from Cluster-Analysis: Application to Real Data

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In a recent publication we investigated an approach to define Metafounders (MF) based solely on the assignments of an unsupervised cluster-analysis of estimated founder-gene-contents by performing a semi-stochastic simulation using true genotypes and a complex pedigree of more than 20k genotyped Fleckvieh (FV) animals. Results of this simulation indicated that an integration of the approach into a Single-Step routine-evaluation in dairy cattle could be feasible and beneficial in increasing validation reliability and reducing inflation of predicted EBV in a trait with a strong genetic trend. In the following we present first results of an adaption of the above approach to data of the current Single-Step routine evaluation for protein yield in German/Austrian Brown-Swiss dairy cattle. To ensure a sufficient level of informativity for the estimation of the gene-contents of pedigree-founders, we used a maximum distance of two generations between the first genotyped (gt) descendants and the ungenotyped (non-gt) pedigree-founders and used this criterion for the truncation of pedigree and phenotypic data. Based on a final pedigree of ~1.4m animals, phenotypic data of ~1.3m animals and ~118k genotypes, we isolated a core-set of ~38k founders related to gt animals, calculated their gene-contents and assigned them iteratively to an increasing number of MF by using a kmeans cluster-algorithm. Pedigree-founders not related to gt animals were assigned to a base-set of 34 MF that was established using standard criteria for the definition of unknown parent groups (e.g. a combination of birth-year and country or origin). Using both assignments, the relationship matrix of MF (Γ) was calculated applying additional restrictions to ensure numerical stability and connectedness to phenotypic data. Finally, Single-Step evaluations with MF using full and reduced phenotypic data (minus 4 years) and standard validation procedures were performed to compare results to the current routine evaluation and to evaluate the impact of using an increasing number of MF. All MF models outperformed current routine-evaluation with respect to validation reliability. However, modelling a (too) low number of MF resulted in a higher bias and considerably more inflation when compared to current routine-evaluation. Modelling ~800 MF was optimal when considering bias, inflation and validation reliability (-1.9, .98, .89) simultaneously. Improvements observed were in good agreement with observations made using simulated data. Given the considerable differences between the current routine-evaluation and the different MF-models investigated, the correlation of breeding values observed in different groups of interest (young/old, gt/non-gt) were high (.96-.98). We observed that the different MF-models largely operate on overall, within and between group variances of EBV. When varying the residual polygenic component in the MF model (~800 MF) the optimum of 25% currently used in routine evaluation was outperformed by variants using 10% and even more when using 5%. By transferring structural information (via Γ) into the conventional relationship matrix, the resulting matrix seems to improve genomic predictions without the need for a large polygenic component in the model. Remarkably, foreign bulls, that are currently integrated into national Single-Step via deregressed MACE EBV validated best when modelling a single MF only (assumption of a homogeneous pedigree-base). MACE, by representing foreign information on national scales, seems to attenuate the stratum characteristics of foreign animals. This suggests that the current integration approach for foreign bulls might not be ideal when switching to MF models.

Estimating variance components and genetic parameters in large genotyped populations

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Accurate genetic parameters require using all data that were available upon selection. However, estimating variance components and genetic parameters in large genotyped populations was infeasible until now. In this study, we present two methods for variance components estimation in large populations under genomic selection. The first is to estimate variance components for the base population, and the second is to compute genetic parameters (heritability and correlation) across time slices to investigate changes over time. The first combines Monte Carlo REML, which uses a Monte Carlo estimator of the trace term of the first derivatives of the restricted log-likelihood to avoid inverting the MME, with an efficient simulation of GEBV under the ssGBLUP framework. This was named MC-ssGREML and implemented in the BLUP90IOD3+ software from the BLUPF90 software suite. The second combines within- and across-trait predictivity formulas with a deterministic formula to predict the accuracy of genomic breeding values, which is named genetic parameters via predictivity (GPP). We tested MC-ssGREML and GPP on real and simulated datasets with up to 330,000 genotyped animals, using single- and multi-trait models. Both methods were compared with REML with (small data) or without (large data) genomic information. For GPP, time slices included three generations, two for training and one for validation. GPP estimates of heritability and genetic correlation closely matched the simulated values, whereas REML was slightly biased. For a dataset with 300,000 genotyped animals, GPP computations took close to one hour for a one-time slice run, including GEBV estimation. GPP had an approximately linear cost with the number of genotyped animals. For MC-ssGREML in a small dataset with 10,000 genotyped animals, variance components were similar to those from ssGREML, and running times were 22 hours for MC-ssGREML and 154 hours for ssGREML. For a dataset with 300,000 genotyped animals, MC-ssGREML required 5 days and 53GB of memory. Both MC-ssGREML and GPP are feasible tools for estimating genetic parameters and variance components in large datasets with many genotyped animals, with the former designed for base-population estimation and the latter for time-slice estimation.

Validating Indirect Genomic Predictions for Low and Moderately Heritable Traits

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Indirect predictions (IP) can serve as a vital tool for modern breeding programs as the number of genotyped animals continues to increase. It provides efficient genomic predictions based on SNP effects and SNP content for the gaps between official evaluation runs, early predictions for young selection candidates, and for commercial animals that may never make it into an evaluation. However, the application and use of IP depend on its reliability, and there is evidence that the method fails to provide satisfactory predictions for traits with low heritability. Our objectives in this study were to investigate whether IP are consistently reliable for traits with low heritability, and whether the choice of core animals in the Algorithm for Proven and Young (APY) can affect IP. Data were provided by the American Angus Association (AAA), which uses IP both as interim evaluations and through the GeneMax advantage program, where they provide genomic percentile scores for commercial heifers. IP were computed for 19 traits with heritabilities ranging from 0.04 to 0.56, using the blupf90 suite of programs. The data included over 15 million phenotypic records with 12 million animals in the pedigree, of which 1.7 million were genotyped. First, benchmark GEBV were calculated with all the available data, including genotypes from validation animals, via single-step GBLUP (ssGBLUP) with APY. Then, data for the validation animals were removed; thus, GEBV were computed based only on information for training animals (all but the validation animals), and back-solved to obtain SNP effects. After that, the IP for validation animals were computed via matrix multiplication using the vector of SNP content and SNP effects. The mean GEBV from the main evaluation (excluding animals having IP) was added to IP, so that IP has similar mean to the benchmark GEBV, thus allowing for direct comparison. Finally, IP for the validation animals were compared to their benchmark GEBV. Traits with high heritability and millions of records produced GEBV and IP that were nearly identical, with Pearson and Spearman correlations greater than 0.99. For lower heritability traits with less data, correlations remained greater than 0.99, except for a trait evaluated using a random regression model, which had a correlation of 0.97. The trends amongst the results were independent of the choice of core animals in APY. Slight differences do exist between GEBV and IP for traits with low heritability, however these are negligible. Thus, demonstrating that IP can be used as interim predictions across traits with varying heritabilities.

Fine-tuning Monte Carlo single-step genomic REML for variance components estimation in large datasets

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As phenotypic and genomic information accumulates over time, estimating variance components (VC) becomes challenging due to computational limitations of approaches that rely on sparse matrix inversion, such as Expectation Maximization (EM) or Average Information (AI) REML. Nevertheless, estimating VC using the same dataset as in the genetic evaluation remains important, as changes in heritability can significantly affect prediction accuracy. The Monte Carlo single-step Genomic REML (MC-ssGREML) estimates VC using either EM or AI-REML, without large matrix storage or inversion. It offers high computational efficiency and low memory requirements for large datasets and complex models involving pedigree and genomic information. Compared to exact approaches, MC-ssGREML approximates the traces of the first derivatives of the restricted log-likelihood (tr(H^(-1)C^(uu))) using an average of quadratic forms, obtained from a sampling process. Although MC-ssGREML is highly efficient, it is preferable to keep the sample size as small as possible, as using hundreds of samples makes the estimation impractical. The feasible number of samples for genetic evaluations (in the order of tens) is limited by a relatively large MC noise near convergence, which can stall the algorithm. The current convergence criteria for MC-ssGREML are limited by MC noise when the sample size is not large enough. The MC noise decreases asymptotically as the number of samples increases. However, this behavior may not be consistent for a smaller sample size. Furthermore, in MC applications, the requirement for MC samples is not constant; more samples are needed towards the end of the estimation process than at its initial stage. In this sense, it is important to select an appropriate number of MC samples to capture the method's convergence nature. This research aims to 1) develop a method for selecting the optimal number of MC samples to improve convergence and keep computing time reasonable, and 2) determine whether a small increase in the number of samples reduces MC noise. We implemented an adaptive sampling scheme that increased the sample count by five whenever the norm of the scaled first derivatives of the restricted log-likelihood (Δc) fluctuated near convergence. We tested it on a four-trait repeatability model with over 210,000 records, 213,297 animals in the pedigree, and almost 16,000 genotyped animals. Starting with five samples, the algorithm ran until it reached 200. Increasing the number of MC samples did not necessarily yield better estimates of tr(H^(-1)C^(uu)) or smoother convergence for complex models. This behavior can be a consequence of the relatively small number of MC samples used in MC-ssGREML, which were insufficient to reduce MC noise significantly. In conclusion, the results indicated that adaptive sampling based solely on Δc did not reduce MC noise or improve the convergence behavior of MC-ssGREML. Addressing the MC sample size challenges and improving convergence requires incorporating MC noise into the adaptive sampling scheme. Nevertheless, MC-ssGREML is a valuable and scalable framework for VCE in large, complex genomic datasets. Further methodological refinements that explicitly address MC noise are expected to enhance its robustness.

Modelling missing pedigree using metafounders and validating genomic predictions in small sheep population

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Genomic selection has been successfully implemented in many livestock species to advance genetic gain. With this, many small-scale breeding programmes for local breeds have begun the transition from traditional selection to genomic selection. However, the success of genomic selection in small-scale breeding programmes can be limited due to lack of organised breeding infrastructure (e.g., lack of widespread use of artificial insemination) and incomplete or incorrect pedigrees. One example of a local breed transitioning to genomic selection is the Pag sheep, a Croatian breed locally adapted for milk and meat production in the Mediterranean climate. To address the mentioned limitations of small, extensively held populations, previous studies have used unknown parent groups (UPG) or metafounders (MF) to model missing relationships. Coupled with leveraging genomic information to correct pedigree errors, UPG and MF showed improved model performance. In that sense, the objective of this study was to analyse bias, dispersion, and accuracy in genomic evaluations for milk yield in Croatian Pag sheep. The dataset consisted of 30,203 whole lactation records for 9,935 sheep across 107 flocks. Genomic information was available for 2,641 sheep of which 2,113 had phenotypes. Initial pedigree was corrected using SeekParentF90 software and then was trimmed to include all phenotyped and genotyped individuals, along with three generations of their ancestors without phenotypic or genotypic information, yielding a total of 12,095 animals. We compared pedigree-based best linear unbiased prediction (BLUP) and single–step genomic BLUP (ssGBLUP), with and without inclusion of UPG and MF. Both UPG and MF were defined based on the year of birth and generation interval. All models were run using blupf90+ program and model comparison was done using the linear regression (LR) validation approach as implemented in validationf90 software, with two validation cohorts: genotyped and phenotyped ewes born in the last generation and genotyped rams of phenotyped ewes born in the last two generations. In our dataset, including genomic information within ssGBLUP improved accuracy compared to BLUP models. The differences in terms of bias and dispersion between the models including UPG and MF, were subtle. While our results highlight the suitability of UPG and MF to improve genetic evaluations in small-scale breeding programmes with missingness in the pedigrees, future studies (e.g., fine-tuning UPG and MF definitions and how they are fit within ssGBLUP framework) are needed to justify their routine use in this population.

Visualization of changes in relationships among traits over time – example in type traits

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Genetic evaluation usually includes many correlated traits. Their evaluation and their use in the selection index rely on the genetic parameters. The genetic parameters are changing, especially under the genomic selection. However, estimating the changes and their interpretation are difficult. The purpose of this study is to present methodology to visualize changes in complex relationships among traits over time. Changing correlations indicate change in relationships among the traits but the multivariate changes are hard to understand only using the genetic correlations. Data in this study were genetic correlations among 18 type traits, which were recorded by the Holstein Association, USA. The correlations were computed by GPP (Genomic Prediction by Predictivity) for years 2010 to 2020 yearly using all available pedigree, phenotypic and genomic information. For year 2010, the reference population included cows born in 2000-2008, and the validation population included cows born in 2009-2011. Subsequent years moved the populations by one year. Using genetic correlations for year 2020, for each trait we computed best prediction equations as indicated by the best fit (R^2) involving 2, 3 and 4 other type traits. Then the coefficients of the prediction equations were recomputed for years 2010-2019. Graphs of the coefficients over time for each trait and 2-4 prediction traits indicated dynamics among the traits and their changes over time. For example, Fore Attachment was a function of Rear Udder Width and Udder Depth (R^2=0.83), with the proportion of Rear Udder Width increasing over time. Adding Dairy Form increased R^2 to 0.86, and replacing Dairy Form by a combination of Stature and Strength increased R^2 further to 0.89. In general, predictions with 4 traits were less stable. GPP can be used to calculate genetic parameters in time slices with data from complete populations. Changes in dynamics of correlated traits can be visualized by prediction coefficients from equations involving 2 to 3 correlated traits.

Establishment of a Nationwide Monitoring System for Genetic and Phenotypic Trends in German Dairy Cattle

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Since the early 2000s, livestock husbandry has gained public attention. Central topics include animal health, welfare, and environmental impacts of dairy cattle farming. In this context, animal breeding has also increasingly moved into the societal spotlight. Although routine evaluations are conducted regularly, there is a lack of nationwide monitoring of breeding-relevant facts at the level of entire populations and complete sets of traits as a standardized basis for scientific and societal discussions. Genetic and phenotypic trends are particularly relevant in this context, as they reflect the results of breeding work as well as management and environmental effects. Current German statistics are published annually, but phenotypic trends are largely limited to milk yield and productive lifespan. Regional milk recording data cover only specific areas. Nationwide genetic trends beyond milk traits are mostly shown as aggregated selection indices, without trends for individual traits. Overall, there is no comprehensive, regularly updated, centrally compiled monitoring system with comparable presentations of genetic and phenotypic trends. For this reason, the project "Monitoring of Genetic and Phenotypic Trends" (MGPT) was initiated in Germany. The aim of this project is to establish a comprehensive, largely automated monitoring system for genetic and phenotypic trends in German dairy cattle breeding. The range of traits considered starts with the set of trait complexes currently included in breeding value estimation, since monitoring genetic trends is based on current estimated breeding values. These trait complexes consist of numerous individual traits which, depending on data availability, are also intended to be integrated into the monitoring system. Particular emphasis is placed on functional traits such as stillbirth rate, calving performance, and health traits. The monitoring initially focuses on three breeds: Holsteins, dual-purpose Fleckvieh, and Brown Swiss. Different breeding goals, genetic parameters, and trait definitions will hamper a direct comparison of trends between breeds. However, comparing phenotypic levels gives information on the selection response of the breeding programs at the national level. Based on the monitoring system, the frequently asked question by farmers and breeders — how breeding values are actually reflected in phenotypes — can be addressed. As an example, breeding values for mastitis (MAS) of 9,028 Fleckvieh bulls born from 2000 onwards and the phenotypes of somatic cell count (SCC) of their first lactation daughters (n = 2,808,115) were analysed. The phenotypes are based on test-day records and were summarised across all test days into a mean SCC per cow. Only bulls with at least 20 daughters with phenotypic records were included in the analysis. In addition, the bulls were grouped into classes from −3 to +3 standard deviations around the mean of their standardized MAS breeding values (mean ≈ 100 points). For each breeding value class, the average phenotypic SCC of the daughters was calculated. The results clearly show that as the MAS breeding values of the bulls increased, the average SCC of their daughters decreased. Using this approach, it would be possible to systematically map all trait complexes included in breeding value estimation and their corresponding phenotypes. This exemplary analysis illustrates the general potential of the monitoring system to make genetic developments traceable at the population level and to demonstrate their corresponding phenotypic outcomes in a transparent manner.

Temporal Dynamics of Major SNPs Identified by Large-Scale Single-Step GWAS in Broiler Chicken Populations

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Genome-wide association studies (GWAS) identify significant associations between single-nucleotide polymorphisms (SNPs) and phenotypes, often reflecting quantitative trait loci (QTL) through linkage disequilibrium (LD). Methods based on marker effects or variance explained may fail to account for uncertainty and LD among markers, so p-values are commonly used to detect significant SNP-trait associations. As allele frequencies and LD patterns shift across generations due to selection, migration, and genetic drift, both QTL effects and their statistical detectability can change over time, influenced by trait heritability, selection intensity, population size, and the number of genotyped animals. Single-step GWAS is widely used but is computationally costly for large datasets because it requires solving mixed model equations. The Algorithm for Proven and Young (APY) offers a sparse approximation of the inverse genomic relationship matrix (G) by splitting the genotyped animals into core and noncore. This study evaluated SNP consistency across generations, identified significant markers using frequentist p-values, assessed the impact of different APY core sizes, and compared marker effect and variance explained approaches. Data from Cobb Vantress Inc. included 1,515,952 broiler chickens, of which 721,041 were genotyped for 46,199 SNPs. Traits analyzed included body weight (BW), breast meat percentage (BM%), and leg score (LS). A multi-trait model accounted for sex, contemporary group, additive genetic effects for all traits, and a maternal permanent environment effect for BW. SNP effects were backsolved from GEBVs and applied in ssGWAS using four APY core sizes, corresponding to eigenvalues explaining 99%x2, 99%, 99%/2, and 99%/3 of the variance of G across generations 1-3, 2-4, 3-5, and 4-6. Additionally, marker effects and their percentage of variance explained were also evaluated for the same time periods. Analyses were performed with the BLUPF90 software suite. GWAS revealed stable SNPs across generations, with BW peaks on GGA1 linked to RING-type domain protein. Similar patterns were observed for BM% on chr5 (ALOX15B, lipid and arachidonic acid metabolism) and chr15 (SLC25A1, mitochondrial citrate transport and negative ferroptosis regulation), and for LS on chr12 (LOC107049643, calcium and phospholipid binding). Mapping resolution improved over generations, likely due to increased genomic information. APY core sizes typically used for genetic evaluations (99% and 99%x2) maintained resolution, whereas smaller cores (99%/2 and 99%/3) reduced precision, occasionally causing peaks to disappear or become less defined. Compared with p-values, marker effect and variance explained approaches revealed diffuse signals, moderate-effect loci and distributed contributions, reflecting limitations that ignore uncertainty and marker correlations. Therefore, major SNPs remained consistent across generations, with p-value analyses identifying clear, stable peaks. APY core sizes used for genetic evaluations maintained mapping resolution, whereas smaller cores reduced precision. Marker effect and variance-explained approaches identify more diffuse signals, providing less informative results than p-values.

Enhancing individual egg number records in group-housed laying hens through integration of behavioral patterns and automated nest system records

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Group housing improves laying hens welfare but complicates individual egg recording necessary for research and breeding, due to shared nest boxes. Automated nest systems Weihenstephaner Muldennest (WSMN, from Big Dutchman) with RFID technology can identify hen visits and potential laying events, yet assignments remain imperfect. Additionally, floor eggs laid outside nests are completely missed, which reduces phenotyping accuracy. We developed an imputation algorithm integrating behavioral data from WSMN records with manual egg counts to improve individual egg number estimation under uncertain identification. Our data came from three German local chicken breeds—Altsteirer (ALT, N=73), Bielefelder (BIE, N=51), and Ramelsloher (RAM, N=86)—housed in group pens each equipped with four automated nest boxes from WSMN. Egg laying was monitored from 24 weeks of age, while the following analysis presented imputation of 69—72 weeks of age, using: 1) WSMN records (hen ID, visit times, egg events); 2) daily manual nest and floor egg counts. Reliable WSMN records were identified based on validity, timing, and frequency criteria. Time offset correction improved match rates between WSMN records and manual counts from 94.6% to 95.2%. The imputation utilises a bayesian update algorithm, iteratively updating egg-hen assignment probabilities using behavioral likelihoods. For each pen-day, trusted assignments from reliable WSMN records matched with manual counts were identified and excluded from imputation. Unassigned eggs (uncertain nest and floor eggs) and candidate hens received a discrete uniform prior, sequentially updated in a bayesian way, using likelihoods from three weighted behavioral features: 1) Individual laying patterns predicted from fitting the reliable WSMN records to a four-state Hidden Markov Model (depmixS4 package), with laying state probabilities served as likelihoods; 2) Short-term laying and nest visitation habits within a ±2-day window, nest likelihoods were accessed by laying and nest visitation frequency incorporating switching penalties, leftover likelihoods were considered for floor eggs; 3) Long-term nest time spent for a hen across the laying period, with proportions served as nest likelihoods. A greedy algorithm assigned eggs to hens with highest posterior probabilities, resolving ties using leftover probabilities or overall laying counts. Cross-validation (3×5-fold) based on trusted assignments evaluated model accuracy. Finally, heritabilities (h^2) were estimated for imputed egg counts via bayesian genomic best linear unbiased prediction (BGLR package) using 52,903 SNPs after quality control. Of 1,721 eggs assigned, 87.5% were trusted matches, 8.4% floor eggs, and 4.1% uncertain nest eggs. RAM had lowest trusted assignments (81.5%) and highest floor eggs (16.6%), while ALT and BIE exceeded 90% trusted eggs and floor eggs below 6.7%. Model accuracy ranged from 0.86–0.93 across breeds. Median h^2 estimates ranged from 0.29–0.33, with RAM highest. Compared to raw WSMN records, imputation increased median h^2 by 0.04, 0.07, and 0.15 for RAM, BIE, and ALT, respectively. The study demonstrates how integration of behavioral data can handle assignment uncertainty while maintaining genetic variance. The flexible structure allows incorporation of additional behavioral or sensor data. However, limitations include reliance on nest visitation, validation based solely on trusted nest eggs potentially overestimating performance. Further, the current prototype only shows slight h^2 improvements in some breeds, which implies the need for optimization of the algorithm over a longer recording phase.

Breeding strategies for pike-perch (Sander lucioperca) in recirculating aquaculture systems in Denmark

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The global production of pike-perch (Sander lucioperca), a freshwater fish species, is increasing. In Denmark, pike-perch are farmed by AquaPri, who produces up to 700 ton per year in recirculating aquaculture systems (RAS). Selective breeding can be a tool to adapt the fish to RAS and to improve efficiency of pike-perch production. This simulation-based study explored different low- cost phenotyping strategies and selection methods for selective breeding in pike-perch. In a basic setup of the breeding program, fish were size graded using a sorting system and selected based on visual inspection of exterior. We modelled this phenotyping strategy by simulating fish size and visual inspection (VI) as two categorical traits. This basic phenotyping strategy was compared with two alternatives. In the first alternative (scaling), fish were weighed using a scale and examined visually for different fish exterior characteristics. Both weight and VI were simulated as continuous, normally distributed variables. In the second alternative, fish were phenotyped using image analysis. The results from the image analysis were used as indicator traits for weight and VI with genetic correlations assumed to be either 0.7 or 0.9, respectively. The basic scenario with sequential selection was compared with multi-trait selection using an aggregated breeding goal. A breeding program for pike-perch in RAS was simulated for 25 years. In a year, there were four batches, in each of which 5 females were mated with 10 males, producing 10 families. In practise a female produces 50k to 60k offspring, but due to computation challenges, we assumed that a batch consisted of 750 fish only, where each of the 10 families contributed 75 fish. Selection was carried out in two rounds at 1 year old fish. In the first round, 150 fish were chosen. In the second round where different phenotyping strategies were applied, 30 fish were selected to become breeders. Sex of the fish was unknown at the time of selection and selected broodstock were used when they were 3 years old. Replacing fish grading with scaling improved genetic gain by 30%. Image-based phenotyping only increased genetic gain when the genetic correlation was 0.9 (19-21%) In addition, multi-trait selection increased genetic gain compared to sequential selection and reduced rate of inbreeding by 2-10%. We recommend adopting scaling and image analysis for phenotyping coupled with multi-trait selection, which requires relatively few operational changes, making it suitable for small breeding programs.

Optimal SNP selection for parentage discovery in large, genotyped populations

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Genotyping is widely used by USA Angus breeders, with over 1.9M animals genotyped, enabling accurate genomic evaluations and allowing pedigree verification and discovery. The American Angus Association uses the ISAG200 panel to verify breeder-submitted pedigrees and to search for parents when pedigree mismatches or missing information occur. Parentage assignment relies on counting the number of opposing homozygotes (NOH), which can become challenging when searching across large populations. If the SNP set is not large enough it could lead to many false positive or negative results and while larger SNP sets improve accuracy, they increase computing time, and exclusion thresholds influence both false positives and false negatives. This study aimed to identify an optimal SNP set and exclusion threshold that balance accuracy, false discoveries, and computing time for parentage discovery in the Angus population. Genotypes for 1.9M animals (54,609 SNP) were available. Two target sets of genotyped trios were selected: 7,775 animals to evaluate eight SNP sets (ISAG200, ISAG554, and custom panels of 200, 554, 1K, 2K, 4K, and 13K SNP), and 74,540 animals to test exclusion thresholds (1–10%) using the 2K SNP panel. Custom SNP sets were sampled from markers with call rate >0.99 and MAF >0.35. Parentage status was determined by counting the NOH. Performance was assessed by the proportion of animals with two parents correctly identified and the distribution of the number of candidate parents found. Panels with ≥1K SNP performed similarly, with >98% of animals having both parents correctly identified, while smaller panels produced more false positives or missed parents. Panels constructed from SNP with high MAF outperformed ISAG panels of equal size. This was largely due to many SNP in the ISAG sets having low MAF in the Angus population. This result demonstrates the importance of selecting markers with high MAF within the target population. In the large target population, exclusion thresholds of 2% and 3% showed the best performance (≥94% correct assignments), while thresholds of 1% or 5% slightly underperformed, and 10% produced many false positives (77.8% correct assignments). Across the 74,540 animals, 98.5% had two parents correctly assigned; only three had more than five candidate parents, all explained by cloned dams. Some animals with zero or one parent identified could reflect sampling or pedigree identification issues and would need to be further investigated to conclude. Sampling SNP within the population using high call rate and high MAF produced the best results. A 1K SNP panel provided optimal accuracy without unnecessary computing load. Because breeder-provided information aids parentage resolution, a threshold that allows a small number of additional candidate parents is preferred. A 3% exclusion threshold was identified as optimal for the American Angus population.

Partitioning the genetic architecture of reproduction traits in Holstein cattle using local heritability

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Reproduction is the prerequisite for lactation and progeny in dairy cattle, but challenging for breeding and genetic improvement. The underlying genetic architecture for such functional and health related traits remains largely unknown and the corresponding heritabilities (h^2) are usually low. Genome-wide association studies (GWAS) are established to identify associated genomic regions even for such low heritable traits, but do not directly quantify the relevance of these regions for the total h^2. However, GWAS summary statistics can be used for additional analyses combined with the linkage disequilibrium (LD) structure of the genome to obtain individual contribution of distinct genomic regions to individual traits. Methodological tools like ρ-HESS or LAVA can be used for this kind of identification and quantification of distinct regions and are also able to identify relevant genomic regions for multiple traits, potentially harboring pleiotropic variants. Subsequently, local genomic regions with higher multi-trait relevance and significance for individual h^2 can be focused on to investigating the genetic architecture. The aim of the present study was to identify and quantify the contribution of individual genomic regions to the total h^2 of selected reproduction traits in German Holstein cattle, using two local h^2 approaches. GWAS summary statistics for seven reproduction traits, namely calving ease direct and maternal (CEd/CEm), metritis (MET), retained placenta (NGV), stillbirth direct and maternal (SBd/SBm) and ovary cycle disturbances (ZYS) were analysed. Up to 250,000 genotyped cows were used for LD reference to estimate 1,906 distinct genomic regions across the 29 autosomes and the X-chromosome. The number of cows for GWAS analyses varied between 110,629 (ZYS) and 192,188 (SBm), and a total of 44,994 SNPs were tested on autosomes and X-chromosome. For all traits between 2 and 33 markers per trait were identified as genome-wide significant after Bonferroni-correction. The total h^2 estimates, derived as sum of all local individual estimates per region and trait differed slightly between the methods ρ-HESS (h^2Hess) and LAVA (h^2LAVA) but were within a similar range (e.g. MET h^2HESS = 0.103, h^2LAVA = 0.087). Across all reproduction traits the h^2 estimates varied between 0.078 (h^2HESS SBm) and 0.127 (h^2LAVA SBd). The relative contribution of each individual region was compared in relation to the overall estimate per method and trait. This comparison revealed similar patterns for the contribution per region and highlighted multiple regions of interest for individual traits. For example, for SBd multiple significant genomic regions were identified distal on chromosome 18 around 58,587 kilobase pairs with both methods, highlighting the relevance of this region for the overall h^2. Finally, both local h^2 approaches identified similar patterns and significant genomic regions contributing to the relatively low overall h^2. This facilitates direct comparisons between traits and allows the assessment of the relative importance of specific regions within and across traits. The obtained results could therefore be used for additional analyses to further pinpoint the relevance of selected genomic regions and investigate their pleiotropic potential across traits.

Large-scale identification of breed-specific alleles in cattle and their potential impact

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Cattle are among the most economically significant livestock, representing a diverse species with two subspecies. Within these subspecies, numerous breeds are adapted to specific environments and production purposes and contain breed-specific alleles. For candidate allele detection, analyses of private alleles are frequently limited to smaller breed panels. Private alleles (breed-specific alleles) represent variants occurring in only one population (breed), regardless of their frequency in that population. In our approach, we analyse a large set of breeds and examine variants that are private to single breeds and their predicted impact to identify potential targets for further studies. For the initial analysis we considered the 1000 Bull Genomes Project and analysed private alleles for 280 breeds across 120 million positions. Breeds were filtered to include only those with at least 15 individuals. For breeds with more than 30 individuals, sample sizes were limited to 30 by random sampling. This process was repeated five times, retaining only the private alleles common to all repetitions. By setting lower and upper boundaries, we aim to obtain representative samples of breed diversity while limiting bias by large breeds on the detection of private alleles. Additionally, comparison was also made against all animals of all smaller breeds as a control group in the identification of private alleles to minimize the false-positive detection of private alleles due to the limited set of breeds passing the filtering step. To identify private alleles, PLINK was used to calculate variant frequencies across breeds, combined with custom scripts for filtering individuals and variants. The identified private alleles were then annotated using SnpEff to predict functional consequences and further filtered for high-impact variants. To investigate potential biological relevance, information from the Animal QTL Database was downloaded and integrated with the private allele data. After filtering for breed size, 62 breeds with 1,544 individuals remain for detection of private alleles. Among breeds, Buryat exhibited the greatest number of private alleles (n = 444,496; 0.4% of all positions), while Holstein had the least (n = 1,479). Initial analyses revealed a total of 2,238 high-impact private alleles across the whole genome. The distribution of private alleles across impact categories differed significantly from that observed in randomly sampled variants. In contrast, only 1,080 high-impact annotations were found among an equivalent number of randomly selected variants, indicating a significant overrepresentation of high-impact variants in private alleles ($p < 0.05$, Fisher exact test), which may point to their potential biological relevance. Angus harbored the greatest number of high-impact private alleles (n = 815), whereas no high-impact private alleles were identified in Holstein Red or Modern Danish Red cattle. Considering the position of private alleles, many are found within regions previously associated with known traits. The categories most frequently overlapping with private alleles were meat and carcass traits, particularly for Angus cattle, and reproductive traits, respectively.

Detection of sex chromosome aneuploidies using SNP array intensity data: an application in pigs.

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Sex chromosome aneuploidies, including XXY (Klinefelter syndrome), XO (Turner syndrome), and XXX (X trisomy), are known to affect reproductive physiology, secondary sexual development, and metabolic traits in humans. However, their prevalence and phenotypic consequences in commercial pig populations remain largely unknown, primarily due to the lack of large-scale detection strategies. We hypothesize that sex chromosome aneuploidies occur at detectable frequencies in pig populations and that they have measurable effects on fertility, growth, and carcass traits consistent with manifestations described in other mammals. This study aims to identify XXY, XO, and XXX individuals using SNP array intensity metrics, Log R Ratio (LRR), and B Allele Frequency (BAF), from ~630K genotyped pigs, and to characterize their performance. Classification will be based on X-chromosome heterozygosity, deviations in mean X-LRR, BAF peak distributions, and Y-chromosome signal intensity. Visual inspection of individual LRR and BAF profiles will further support classification accuracy. Normal euploid males (XY) are expected to show near-zero heterozygosity across the X chromosome, a baseline X-LRR, and a BAF clustering at 0 and 1, consistent with a single X copy, and a clear Y-chromosome signal. In contrast, XXY males are expected to present increased X-chromosome heterozygosity relative to XY males due to the presence of two X chromosomes, a positive shift in mean X-LRR consistent with an additional X copy, BAF clustering compatible with disomic states (peaks at 0, 1/2, and 1) across most loci, and preserved Y-chromosome signal. XO females are anticipated to exhibit minimal or absent heterozygosity across the X chromosome, markedly reduced X-LRR (reflecting monosomy), BAF clustering at 0 and 1 (consistent with a single X copy), and no detectable Y signal. Conversely, XXX females are expected to have elevated mean X-LRR consistent with trisomy, BAF peak distributions at 0, 1/3, 2/3, and 1 across loci with greater heterozygosity, and absence of Y signal. In the first screening, 0.003% XO and 0.014% XXX among total females, and 0.2% XXY among total males, were identified. Based on evidence from human and comparative mammalian studies, we expect XXY males to show impaired spermatogenesis, an increased incidence of testicular hypoplasia, and reduced fertility. We also expect an altered androgen–estrogen balance associated with supernumerary X-linked gene dosage, which may influence muscle deposition and fat metabolism, potentially leading to differences in body weight and backfat thickness compared with euploid males. For XO females, we hypothesize (near-)complete infertility due to gonadal dysgenesis, although some individuals may display estrous behavior without successful conception. For XXX, we predict partial reproductive impairment, with subfertility manifested as reduced farrowing rate or smaller litter size. Hence, for the next steps of the analysis, phenotypic effects will be evaluated through case-control comparisons within genetic line, birth year and month, and farm to isolate chromosomal effects from environmental and management confounders. In the future, we hope that SNP-intensity–based screening will enable routine, large-scale identification of sex chromosome aneuploidies during the selection of breeding candidates, thereby improving reproductive efficiency management in pig production.

Estimation of semen variability phenotypes in boars used for artificial insemination

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The swine industry relies strongly on the regular and reliable production of high-quality fresh semen. In production centers of semen doses, it is of utmost value to predict the number of doses that can be produced weekly from a boar. Therefore, boars with lower variability in semen quantity are desired. The objective of this study was to estimate new phenotypes for boar-level variability in semen using the residual variance of these traits. Ejaculates were collected between 2021 and 2026 and evaluated using a Computer-Assisted Sperm Analysis (CASA) system. The phenotypic dataset included records from 188,852 ejaculates collected from 2,373 boars from a synthetic sire line. Pedigree information included 9,289 animals spanning 15 generations. We considered semen quantity (number of sperm cells, concentration and volume of the ejaculate) and sperm quality parameters (sperm total and progressive motility of fresh semen and total morphological abnormalities). We estimated residuals using a univariate analysis with a repeatability model accounting for fixed effects at semen collection. The boar-level residual variance was calculated and transformed using an inverse normal transformation. We used a univariate model to estimate genetic parameters for boar-level residual variance and a bivariate model to estimate genetic correlations with boar-level mean of the trait. Results indicate that boar-level semen quantity and sperm quality variability phenotypes (residual variance) have moderate heritabilities (0.15 – 0.27) and can be improved by means of selection. For semen quantity traits, the boar-level variability has a positive genetic correlation with boar-level mean of the trait (0.52 – 0.74). For sperm quality traits, the boar-level variability is moderately or not correlated with boar-level mean of the trait (-0.13 – 0.43). These results indicate that both semen quantity and semen quantity variability should be included in the breeding program, in order to allow for increased genetic gain on semen quantity while preventing selection for boars with less predictable number of sperm cells per ejaculate.

easybreedeR: An integrated R shiny application for breeding data analysis

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Abstract Phenotypic, pedigree, and genomic data form the foundation of modern animal and plant breeding. Before the analysis process, these datasets must undergo biological and statistically rigorous quality control to ensure accurate estimation of genetic parameters and breeding values. However, the process of preparing and validating the breeding datasets, as well as the generation of parameter files for genetic and genomic evaluations (e.g., using the BLUPF90 family of programs), often involves fragmented tools and manual scripts. This workflow is usually time-consuming and prone to errors, which may become a major barrier for new users; especially those without extensive programming experience. To address this gap, we introduce easybreedeR, an integrated online interactive toolkit for the purpose of breeding dataset verification, quality control, and the automatic generation of parameter files used by the BLUPF90 programs. By standardizing data structures and model specifications, easybreedeR reduces manual errors, improves reproducibility in genetic and genomic evaluations, and significantly reduces the challenges for new researchers interested in the field of breeding. The easybreedeR toolkit also provides clear, comprehensive summaries of phenotypic, pedigree, and genomic data, offering a user-friendly environment that bridges data preprocessing and genetic evaluation. By integrating quality control and automated parameter-file generation within a single platform, easybreedeR streamlines complex breeding workflows and makes advanced analyses more accessible to researchers and breeding practitioners. Availability: easybreedeR is developed primarily in R with Rcpp and implemented as an interactive Shiny application. The development version is maintained at https://github.com/rojaslabteam-rgb/easybreedeR. An online version has been deployed on shinyapps.io and is available at https://vb6clt-huangyi-tang.shinyapps.io/easybreeder-suite/.

Towards a Stillbirth Monitoring System for German Holstein Dairy Cattle in the MGPT Project

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Stillbirths in dairy cattle do not only have economic consequences but are also highly relevant from an animal welfare perspective. The literature associates factors such as under- and over-conditioned transition cows, male sex, primiparous calvings, dystocia, as well as twin births and obstetrical interventions with increased stillbirth rates. In addition, various infectious agents may influence stillbirth rates depending on regionally differing epidemiological conditions, e.g. BTV-3. Known genetic defects are mostly recessive mutations that are widespread within the population. Large datasets of genotyped healthy cattle enable the identification of haplotypes carrying unknown lethal defect alleles due to missing homozygous animals in the population. However, identifying such alleles requires a certain frequency within the population and targeted monitoring to allow statistical detection. Rare and potentially novel mutations are therefore generally unknown and may cause considerable damage until they are discovered, particularly as they are often investigated systematically only at a later stage. Given this complexity, the causes of stillbirth are heterogeneous in aetiology and require comprehensive and differentiated recording of relevant influencing factors. On the other hand, specific challenges arise in collecting such data, as stillborn calves have no economic value for farmers, and in-depth investigations or genotyping are therefore performed only in exceptional cases. In this context, the project MGPT (Monitoring of Genetic and Phenotypic Trends) aims to establish a monitoring system for stillborn calves in the German Holstein Friesian dairy cattle population. Specific phenotypic characteristics recorded include time of death (intrapartum or postpartum), the course of parturition, fetal development, externally visible abnormalities or malformations, chest circumference, birth weight, sex, and the body condition of the dam. In addition, photographs can be taken to allow subsequent estimation of calf size and retrospective assessment of abnormalities. For analysis, the recorded data are merged with routine data collected by vit (IT Solutions for Animal Production), such as previous calvings, lactation number, breeding values, genetic characteristics, insemination data, and any prior stillbirths. This enables classification according to potentially common causalities. The phenotyping is accompanied by SNP genotyping of stillborn calves, while their parents are routinely genotyped. Since the beginning of 2025, 87 farms with a total of approximately 16,000 cows have participated. As of 28 February 2026, a total of 556 stillbirths have been recorded. The monitoring will continue to enlarge the reference population for the identification of phenotypic and genotypic causes of stillbirth and to develop a prospective risk monitoring system for defective alleles, enabling early breeding intervention.

Monitoring of Genetic and Phenotypic Trends in German Dairy Cattle – Project MGPT

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In recent years, dairy cattle breeding has undergone substantial changes. Alongside technological innovations such as genomic selection, breeding objectives have shifted considerably. Functional traits, animal health, animal welfare, and aspects of resource and climate efficiency are increasingly being prioritized, thereby complementing established breeding objectives in a sustainable manner. Despite these adaptations, however, dairy cattle breeding is facing increasing scrutiny in public perception and political discourse. A robust evaluation of trait development in dairy breeding requires a comprehensive analysis of both genetic and phenotypic trends. These trends are instrumental, as they reflect the cumulative effects of selection decisions as well as the influence of management practices and environmental conditions over time. In Germany, such assessments are limited and address only parts of the dairy cattle population. The collaborative project "Monitoring of genetic and phenotypic trends" (MGPT) aims to close this gap by developing a nationwide monitoring system covering breeding-relevant parameters at the level of entire populations and comprehensive trait sets. Initially, the monitoring will focus on the breeds Holstein, Fleckvieh, and Brown Swiss, with a long-term perspective of gradual expansion to include additional populations, particularly traditional and endangered breeds. Special emphasis will be placed on animal welfare- and environment-related traits, thereby establishing a scientific basis for assessing dairy production systems in the context of climate change. Ultimately, the goal is to establish a centralized, publicly accessible information platform that provides robust data to support evidence-based decision-making for policymakers and industry stakeholders, while simultaneously offering an objective foundation for societal discourse on dairy farming. Furthermore, methodological frameworks will be developed to enable the routine monitoring of stillbirth incidence, a trait of particular relevance to animal welfare, and to facilitate a deeper investigation of its underlying causes. At present, the recording of stillbirths does not differentiate between calves born dead and those that die within the first 48 hours postpartum. A more precise phenotypic definition, together with improved knowledge of both genetic and non-genetic determinants and a more detailed recording of environmental influences, may contribute to further reductions in stillbirth rates and the incidence of severe calving events. In the long term, this approach aims to establish the foundation for an early warning system that supports timely breeding interventions. For instance, it would enable the identification of deleterious mutations associated with increased defect rates and adverse welfare outcomes before they become widespread, thereby preventing costly counter-selection measures and potential losses in genetic diversity. The resulting insights will be systematically incorporated into the monitoring framework.

High-Sensitivity Detection of Genome-Edited Pigs and Cattle by CAST-PCR

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With the development and increasing use of new genomic techniques (NGTs) for genome editing to generate novel genetically modified animal products, new detection approaches must be developed. Because NGT-modified organisms may differ from non-modified strains by as little as a single nucleotide mutation and do not necessarily contain foreign DNA, a high level of specificity and sensitivity in mutation detection is required. To address this challenge, and as a partner in the European project DETECTIVE (Detection of NGT products to promote innovation in the European Union), PROGENUS has developed a CAST-PCR (Competitive Allele-Specific TaqMan PCR) approach to detect a two-nucleotide deletion in the bovine PMEL gene and in the pig CD163 gene. This technique is based on target-specific amplification and detection using TaqMan qPCR, involving primer competition between the wild-type and mutated sequences. Preliminary tests highlight a limit of detection (LOD) under 10 copies. In addition to its sensitivity, the CAST-PCR detection test developed is robust, easily applicable in routine laboratories without the need for specialized equipment, and cost-effective. These development efforts will provide new analytical tools enabling the detection of NGT-introduced mutations in derived products.

Genetic investigation of the boar taint compounds androstenone and skatole using SNP chip data and whole genome sequencing data in dam lines

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Due to consumers' rising animal welfare concerns, surgical castration without anaesthesia is discussed critically in Europe and was banned in Germany in January 2021. Besides surgical castration under anaesthesia, fattening entire boars is a promising alternative. However, this method is restrained by the slight risk of boar-tainted meat and an unfavourable fatty acid composition of the back fat. To lower this risk, breeding organizations have started breeding against boar taint, based on either records from human sensory scoring (SENS) systems or the chemical components androstenone (AND) and skatole (SKA). Previous studies have shown that these phenotypes have moderate (SENS) to high (AND) heritabilities ranging between 0.15 to 0.52. It has been demonstrated that AND exerts an inhibitory effect on cytochrome CYP2E1 activity, leading to an antagonist effect on the SKA degradation in the liver. The genetic correlation between AND and SKA is moderate (rg= 0.42 – 0.57). Another potential risk is the possibility of an antagonistic relationship between AND and endocrine fertility parameters (EFP). Quantitative genetic analyses of maternal nucleus lines of a German breeding organization showed only a weak genetic correlation between AND and different fertility parameters (rg= 0.03 to 0.36), but a strong genetic correlation between AND and the EFP testosterone (rg= 0.62 and 0.83). Based on routinely collected SNP chip data (Illumina PorcineSNP60 BeadChip), our group performed genome-wide association studies for boar taint components as well as for fertility parameters and EFP. The studied data originated from the project G-I-FER (supported by funds of the Federal Ministry of Food and Agriculture based on a decision of the Parliament of the Federal Republic of Germany via the Federal Office for Agriculture and Food under the innovation support programme; FKZ: 2817904115). In these studies, 15 QTL for AND and 28 QTL for SKA in Landrace (LR) and Large White (LW), and a few suggested significant QTL for fertility parameters, and EFP were identified.Therefore, the objective of the current study is to characterize genomic regions associated with AND and SKA based on imputed whole-genome sequencing (iWGS) data of LR and LW populations. The SNP chip genotypes of phenotyped animals for AND and SKA were imputed based on a subset of 57 animals with WGS data. These reference animals were carefully selected in a combined haplotype- and pedigree-based approach within the framework of a project aiming to improve survivability and immunocompetence. The WGS data originated from the project pigFit (supported by funds of the German Government's Special Purpose Fund held at Landwirtschaftliche Rentenbank; FKZ28-RZ-3-72.038). Animals are assumed to capture the genetic structure of the LR and LW populations.Target regions based on our previous studies and the AnimalQTLdb include SSC5 (21.17 – 24.5 Mb) and SSC6 (53.16 – 81.9 Mb) for AND and SSC7 (71.0 – 75.04 Mb) and SSC14 (140.6 – 141.7 Mb) for SKA. Imputed WGS data enables fine mapping of the regions associated with boar taint compounds. Therefore, a deeper insight into the genomic background of AND and SKA is assumed, and these insights may be incorporated in the genomic selection of boars with reduced boar taint potential.

How Pure is Pure Breeding? A Case Study of a commercial German pig breed

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Traditional pig breeds, such as the German Edelschwein (DE), are mainly preserved through purebreeding to maintain breed identity. Herdbooks regulate inbreeding, kinship, and crossbreeding. Purebreeding programs may use targeted crossbreeding, but the extent and effects of introgression remain poorly studied. Pedigree-based analyses may underestimate population trends, especially inbreeding, due to incomplete ancestry information, whereas genomic data provide a more independent assessment.The DE breed has been independent since 1904 and, alongside the German Landrace, is a major maternal line in commercial crossbreeding in Germany. As a case study, introgression events in DE were analyzed using pedigree and genomic data. Objectives were (1) to quantify introgression dynamics and population metrics (effective population size (Ne), inbreeding) from pedigree and (2) to assess how well pedigree-based estimates of genetic contributions reflect actual genomic breed composition. The pedigree dataset comprised 77,999 pigs born between 1978 and 2025 after data cleaning. Pedigree analyses used R (optiSel), PEDIG, and POPREP. Genomic data from DE and Large White pigs were evaluated using an Illumina SNP array (~80K markers BeadChip). 234 animals per breed (DE and Large White) born between 2010 and 2023 were available. Genomic characterization was performed via ADMIXTURE analysis and principal component analysis (PCA).For animals born within the last ten years, pedigree completeness was 88.5% in the first generation and 57% by the sixth. The average maximum generation depth was 14.08, with overall completeness of 0.13; the generation interval averaged 2.1 years.DE population size has fluctuated. Between 2005–2007, Ne declined to 562 with inbreeding coefficient F = 0.15. From 2010 onwards, inbreeding increased alongside improved pedigree completeness, suggesting trends may partly reflect data depth. In 2016, Ne peaked at 1,276 with F = 0.03; since then, population size has declined.Pedigree analysis identified 476 animals with parents from different breeds, mainly Large White; 257 crossbreeding individuals were born between 2002–2006. Consequently, 22% of DE exceeded 50% foreign breed proportion in 2006. In the current reference population (n = 2,315), foreign contributions remain in ~30% of animals but each <1%. The PCA of the genotype data shows changes over time that may reflect introgression.These results show that even formally managed purebred populations may contain contributions from other breeds. Classifying animals with up to 50% foreign ancestry as members challenges the concept of genetic purity. The results aim to evaluate whether short-term introgression events may lead to long-term changes in the genetic composition of the DE population and, consequently, raise questions about how much of the original DE genetic background remains.

Local ancestry aware genome-wide association study of dairy traits using both purebred and crossbred data

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The availability of phenotypes on crossbred individuals is increasing. These phenotypes can increase the number of observations when a trait can be assumed to be the same in crossbreds and purebreds. The crossbred phenotypes should be analyzed with specialized multi-breed genome-wide association studies (GWAS) and genomic prediction models to account for breed differences in allele frequencies and allele substitution effects. These specialized models are generally referred to as breed-of-origin (BOA) models, and they are linear mixed models that provide estimates of breed-specific marker effects by forming breed-specific model matrices for genomic information using inferred ancestry of each marker allele. Such local ancestry aware models have been shown to improve the accuracy of genomic predictions in cattle and increase the power to identify influential loci in admixed human populations, but they have scarcely been used for GWAS of livestock populations. We hypothesized that combining purebred and crossbred data in a local ancestry aware GWAS improves the statistical power over within-population GWAS. This research question was investigated using phenotypes adjusted for fixed effects for fat, protein, and milk yield in the Nordic breeds for Holstein (N=48,647), Jersey (N=27,503), Red Dairy (N=9,991), and Crossbreds (N=6,517). All animals with adjusted phenotypes had SNP information from 41,238 marker loci. The local ancestries were inferred for crossbred animals using the purebred animals as reference animals. We performed one GWAS for each breed with data from only purebred individuals, and we performed a combined GWAS with data from both purebred and crossbred individuals. The GWAS models were fitted with the Tractor software, where inferred local ancestry is used to decompose each SNP genotype into breed-specific dosages, and breed-specific allele substitution effects are defined as fixed regressions on these dosages. We corrected for multiple tests using the Bonferroni correction with P(<0.05) and for 41,238 tests. In the purebred analyses, we found 8,347-12,289 influential loci for Holstein, 4,672-6,788 for Jersey, and 1,462-4,193 for Red Dairy. For these loci, more than half of the P-values were smaller in the combined analysis than in the purebred analyses (Holstein: 57-60%, Jersey: 71-84%, Red Dairy: 53-72%). Furthermore, the combined analysis found more significant loci than the purebred analyses across all traits (Holstein: 130-175 loci, Jersey: 367-671 loci, Red Dairy: 314-645 loci). This increase may partly reflect the larger sample size in the combined analysis, and further work is needed to separate sample size and model effects. In conclusion, the results indicate that the local ancestry aware GWAS was able to utilize genetic signals in the crossbred data in combination with the purebred data to achieve greater statistical power than the per-breed GWAS with the caveat that the results should be verified on simulated data for certainty.

Genetic Markers Associated with Slaughter Yield in Intensively Fattened Lambs Raised in Latvia

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Improving carcass performance is a primary objective in meat sheep production systems, as slaughter yield directly affects farm profitability. Genetic improvement of this trait offers a sustainable approach to enhancing production efficiency in local breeding populations. This study evaluated associations between selected candidate gene polymorphisms and slaughter yield in intensively fattened lambs raised in Latvia. The experimental group consisted of 160 lambs, including 59.5% Latvian Dark-Head animals, managed under standardized feeding and housing conditions. Slaughter yield averaged 44.45 ± 2.52% and was recorded at 149.65 ± 14.26 days of age. Fifty-seven SNPs across eight biologically relevant candidate genes were genotyped. Association and regression analyses identified 23 SNPs (40.35%) significantly related to slaughter yield. The strongest associations were observed for variants in the UPC2 and MTOR genes. For UPC2, lambs homozygous for the common allele demonstrated higher slaughter yield, although this genotype occurred at low frequency. In the MTOR gene, superior performance was observed in both homozygous common and heterozygous genotypes, which together represented approximately 25% of the population. These findings indicate that specific genomic variants may be incorporated into breeding strategies aimed at improving carcass efficiency in Latvian sheep populations. The results provide a foundation for marker-assisted selection targeting economically important production traits. Acknowledgements: This study was supported by the University of Latvia Postdoctoral Project No. 1.1.1.9/LZP/1/24/027 and by the Latvian Council of Science, Latvia, project No. LZP-2021/1-0489.

Semen Cryopreservation as a Conservation Strategy for the Native Species Salmo ghigii in Sardinia: Results on Fertilization and Embryonic Development

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The Mediterranean trout (Salmo ghigii) represents one of the most vulnerable native salmonids in Southern Europe, severely threatened in Sardinia by extensive introgressive hybridization with non-native domestic strains. Conservation programs rely on supportive breeding of genetically validated wild spawners; however, reproductive asynchrony among breeders frequently limits fertilization success and reduces effective population management. In this context, semen cryopreservation may represent a strategic tool to decouple gamete availability and enhance long-term genetic conservation. During the 2024–2025 reproductive season, semen was collected from three wild males, pooled to maximize genetic representation, diluted (1:4) in Fish Freezing Medium, and cryopreserved in 0.25 ml straws using a controlled-rate freezing protocol (–13.6°C/min to –140°C). Post-thaw sperm quality parameters were compatible with fertilization, with total motility ranging from 16–22% and progressive motility from 10.7–16.2%. In the subsequent 2025–2026 breeding season, eggs from sixteen genetically confirmed females were equally divided and fertilized using either fresh or cryopreserved semen under standardized hatchery conditions. A total of 1069 eggs were fertilized with fresh semen and 1127 with cryopreserved semen. Embryo survival to release reached 830/1069 (77.6%) in the fresh group and 422/1127 (37.4%) in the cryopreserved group, while embryo mortality was 3.4% and 8.3%, respectively. Statistical analysis (Welch's t-test) showed no significant differences between treatments in fertilized egg number or mortality rate ($p > 0.05$). Conversely, viable embryo output and survival percentage were significantly higher in the fresh semen group ($p = 0.0017$ and $p < 0.001$, respectively), confirming a measurable efficiency gap between treatments. Despite the reduced performance of cryopreserved semen, it generated 422 viable juveniles, contributing to the release of 1252 individuals into native habitats. These results demonstrate that semen cryobanking, although less efficient than fresh fertilization, provides a substantial and operationally reliable contribution to recruitment, reduces the need for prolonged broodstock maintenance, and enhances flexibility in genetic management strategies. Overall, cryopreservation emerges as a robust complementary conservation tool capable of mitigating reproductive asynchrony and strengthening long-term preservation efforts for S. ghigii in Sardinia.

Characterization of Missense Mutations in Acute Phase Proteins in a Cellular Model for Chicken Amyloid Arthropathy

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Amyloid arthropathy (AA) in laying hens is a form of amyloidosis characterized by deposition of amyloid in large joints, which consists mainly of Serum Amyloid A (SAA). The disease is induced by bacterial infection, most frequently by Enterococcus faecalis (E. faecalis), which is the most frequent isolate in field cases and a major multidrug-resistant poultry pathogen. Brown-egg-laying hens (BL) are affected, whereas white-egg-laying hens (WL) appear resistant, with a line-specific immune system (cell-mediated in WL vs humoral in BL). We identified and functionally characterized a dysfunctional missense mutation in SAA (SAA.R90S) in WL, and we discovered six associated missense variants in the fibrinogen alpha chain (FGA) gene in BL. While SAA is a key driver of AA, fibrinogen has not previously been implicated, despite being amyloidogenic and capable of binding free SAA to promote amyloid formation. We therefore investigated whether SAA and FGA variants affect cellular programs relevant to AA. Stable cell lines overexpressing SAA.WT, SAA.R90S or empty vector control were co-transfected with fibrinogen subunits (FGA.WT or FGA.MUT [carries six missense mutations], FGB, FGG) co-expressing distinct fluorophores via P2A. Triple-positive cells were FACS-isolated to obtain cell populations that express the fully functional fibrinogen complex. These cells were then subjected to RNA-sequencing for differential gene expression analysis. Reads were aligned to the reference genome, followed by differential gene expression and gene set enrichment analysis (GSEA) using R (DESeq2, clusterProfiler). FGA overexpression (MUT vs Cntrl or WT vs Cntrl) elicited hundreds of shared differentially expressed genes (DEGs) across SAA backgrounds (435 for FGA.MUT, 458 for FGA.WT), reflecting metabolic stress and cellular stress responses (oxidative phosphorylation, ribosome, peroxisome, DNA replication). Venn diagrams further revealed SAA variant-specific signatures, with a stronger effect of SAA.WT on overall gene expression (129 vs 40 for SAA.R90S), enriched for developmental and regulatory pathways, including TGF-β signalling, cell cycle, and Hedgehog signalling. In contrast, SAA.R90S preferentially enriched proteasome and MAPK signalling, and integrin/ECM-related pathways, consistent with activation of protein quality control and enhanced stress adaptation. FGA.WT and FGA.MUT induced largely overlapping transcriptional programs across SAA backgrounds, with only minimal differential gene expression between variants. These findings indicate that SAA variants qualitatively shape the amyloid-relevant cellular response, while FGA variants amplify metabolic stress, supporting genetic contributions to line-specific AA susceptibility. Complementary proteomic studies are planned to dissect context-dependent SAA-fibrinogen interactions driving disease.

De-novo assembly of a high-quality, haplotype-resolved genome assembly for the Ugandan Ankole cattle

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The Ankole is a long-horned Sanga cattle breed (Bos indicus africanus) native to Uganda and widely distributed across sub-Saharan Africa and beyond. It is valued for disease and parasite tolerance, adaptation to the harsh tropical environment and unique cultural significance. The ARS-UCD2.0 cattle reference genome is based on a Hereford cow (Bos taurus taurus), and introduces reference bias in indicine cattle studies. To better understand the distinctive features of the Ankole, we generated a haplotype-resolved assembly (PacBio HiFi + Hi-C) to improve discovery of previously unresolved variants of this breed. PacBio SMRTbell and Dovetail Omni-C libraries were prepared from genomic DNA and sequenced on PacBio-Sequel (~67X) and Illumina-HiSeqX (~30X) platforms respectively. Draft assemblies were created with Hifiasm, cleaned with blobtools2, and haplotigs removed with purge_dups3, to produce two haplotype assemblies that were scaffolded with Omni-C reads using HiRise and super-scaffolded using PAFScaff. Assembly quality was assessed using BUSCO with eukaryota_odb12 data. For validation, whole genome sequence reads from 19 Ankole cattle were mapped to 29 autosomes of each haplotype assembly and the reference genome using BWA. Mapping rate was normalized by assembly length and compared with paired t-test. Variants were called using Deepvariant, summarized with bcftools and compared with the Wilcoxon signed-rank test. Haplotype-resolved assembly sizes were 3.20 Gb (963 scaffolds, contig N50: 68 Mb, scaffold N50: 75 Mb) for haplotype1 (H1) and 2.99 Gb (887 scaffolds, contig N50: 67 Mb, scaffold N50: 86 Mb) for haplotype2 (H2). Respective BUSCO completeness was 98.4% and 97.7% with GC content of 43.86% and 43.9%. Reference-guided anchoring and scaffolding yielded 30 super-scaffolds for each assembly (H1:2.81 Gb, scaffold N50:108.84 Mb and H2:2.60 Gb, scaffold N50:108.11 Mb) corresponding to 29 autosomes and sex chromosomes (H1:X, H2:Y). Mapping results showed significantly higher normalized read density when aligning to the H1/H2 assemblies compared to the reference (H1/Ref: +21,835 reads/Mb, p-value (p): 9.48×10^{-22}, H2/Ref: +23,412 reads/Mb, p: 7.55×10^{-22}). Additionally, H2 presented significantly higher read density than H1 (+1,576 reads/Mb, p: 9.48×10^{-20}). Variant discovery significantly improved relative to the reference with H1/H2 detecting more high-quality SNPs (H1/Ref: + 165012, $p = 3.78\times10^{-3}$, H2/Ref: +233441, $p = 7.63\times10^{-6}$, H2/H1: +68428, $p = 4.82\times10^{-2}$) and less INDELs (H1/Ref: -102108, $p = 5.35\times10^{-11}$; H2/Ref: -95316, $p = 7.63\times10^{-6}$). Both haplotype assemblies showed increased alignment relative to the reference assembly, with Hap2 presenting superior mapping and variant detection rate than Hap1. The assembled haplotype-resolved Sanga genomes enhance variant recovery, reduce reference bias and capture structural variation more effectively than the reference fostering studies on adaptation, resilience, and the genetic improvement of native African cattle.

Influence of genetics and rumen microbiome on bovine milk vitamin B2 and B12

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Bovine milk is a key source of essential nutrients, providing all 13 known vitamins, including riboflavin (B2) and cobalamin (B12), which are vital for numerous biochemical processes. Because humans cannot produce these vitamins in sufficient amounts, they must be obtained through the diet, and inadequate intake can lead to various health problems. Vitamin B_2 in dairy cows originates from both dietary intake and ruminal microbial synthesis by bacteria, fungi, and protozoa, though much is modified in the rumen before absorption in the proximal jejunum via specific transporters. Vitamin B_{12}, in contrast, is synthesized exclusively by rumen microbes through a cobalt-dependent pathway involving B_{12}-producing bacteria and archaea. The hypothesis is that both host genetics and rumen microbiome composition contribute to variation in milk vitamin B_2 and B_{12} levels, with genetics expected to have significant influence. Microbial effects are anticipated to be stronger for vitamin B_{12}, as it is exclusively synthesized by rumen bacteria, whereas vitamin B_2 has both dietary and microbial origins. Vitamin B2 and vitamin B12 content were determined in 281 milk samples of Holstein cows from 3 commercial farms. These cows were genotyped (37,458 SNP markers available after filtering), and the rumen microbial composition was determined using 16S rRNA sequencing. Vitamin concentrations were transformed (square root for B_2, $\log_{10}$ for B_{12}) for normality. Mixed models estimated heritability and microbiability, with parity and days in milk (DIM) included as fixed effects, and G-matrix and M-matrix as random factors. The study evaluated predictive models using genetic, microbial, and combined data. Results showed moderate heritability for B2 (0.45 SE: 0.19) and B12 (0.37 SE: 0.22), indicating substantial genetic control. Microbiability was negligible for B2 (0.00 SE: 0.04) but modest for B12 (0.09 SE: 0.06), reflecting its microbial synthesis in the rumen. Prediction accuracy of milk vitamin B2 content based on the full model resulted in: GEBV: 0.58 and MEBV 0.51, whereas prediction accuracy of milk vitamin B12 content resulted in: GEBV: 0.68 and MEBV: 0.60. Preliminary results show that genetics plays a role in vitamin B2 and B12 content of bovine milk, and that the rumen microbiome has a larger influence on B12 compared to vitamin B2 as expected according to scientific literature.

Near teleomere-to-telomere assemblies of Swedish commercial dairy cattle breeds, Swedish red- and white, Holstein and Jersey

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The creation of reference-quality genome assemblies based on long-read platforms is now so cost-effective breed-based reference assemblies are now feasible. For this project family trios of Swedish Red and White, Holstein (with a small proportion of Swedish lowland cattle) and Jersey were sequenced with the goal of trio-binning based de novo assembly. This makes it possible to partition long reads from an offspring into haplotype-specific sets. Each haplotype is then assembled independently, resulting in a complete diploid reconstruction. Recent advances in long-read sequencing technologies have made it possible to generate near telomere-to-telomere (T2T) assemblies, although highly repetitive genomic regions still present challenges. The objective of this study was to evaluate long-read sequencing strategies for generating highly contiguous de novo genome assemblies of Swedish dairy cattle. Family trios representing the Swedish Red and White, Holstein and Jersey breeds were sequenced using PacBio Revio and Oxford Nanopore PromethION. The daughters were sequenced on the PacBio Revio platform across multiple SMRT cells, generating more than 100 Gb of HiFi reads per individual, while each parent was sequenced to approximately 60 Gb. De novo assemblies were generated using the trio-aware assembler Hifiasm version 0.25.0, producing several graph genome assemblies using different combinations of HiFi and ONT ultra-long read data, with the largest contig reaching 160 Mb, corresponding to chromosome 1. The total assembly size for the Swedish Red and White genome was approximately 3.1 Gb. This assembly consisted of approximately 1,100 contigs, with a k-mer quality score of 61 and a BUSCO completeness score of 98% for single-copy genes (0.73% duplicated genes) using the Artiodactyla dataset. To further evaluate assembly completeness, satellite repeat structure was analysed with a focus on centromeric and telomeric regions. Given the acrocentric structure of Bos taurus autosomes, repeat analyses show chromosomes remain T2T-like rather than fully telomere-to-telomere assembled. In addition, daughters were sequenced on the Oxford Nanopore PromethION platform, generating approximately 90 Gb of ultra-long reads for Swedish Red and White cattle 44 for Holstein and 51 for Jerse, Ultra-long reads (>100 kb) represented 916 Mb, 442 Mb, and 511 Mb for Swedish Red and White cattle, Holstein and Jersey respectively. Reads exceeding 300 kb comprised ~6 Mb in Swedish Red and White cattle but ~1 Mb in Holstein, indicating a substantially stronger ultra-long tail in the former dataset. These results demonstrate that current long-read sequencing strategies combined with modern assemblers such as Hifiasm can produce highly contiguous cattle genome assemblies approaching T2T quality, providing access to streamlined bioinformatics software able to generate improved genomic resources for dairy cattle research. The inclusion of Ultra Long read-data from the Oxford Nanopore will further improve the assemblies but are only likely to increase completeness by providing scaffolding in long repetitive regions. In the next step graph genomes by the addition of 8 individuals each of each breed with raw yields of 36 to 71 Gbp generated by Pacbio Revio.

Accuracy of Genomically Enhanced Breeding Values in South African Holstein Cattle for Milk Production Traits

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Genomically enhanced breeding values (GEBVs) derived from single-nucleotide polymorphism (SNP) markers can be estimated for animals without phenotypes. However, there is a low prediction accuracy in South Africa Holstein cattle due to the limited number of genotyped animals, furthermore, little is known about how the size of the validation population affects GEBV reliability. Therefore, the objective of this study was to evaluate the accuracy of GEBVs in SA Holstein cattle. The dataset consisted of lactation records of 354 228 Holstein cows and 541 325 pedigree records. Each cow could have up to three lactations in the dataset. Only 1 473 of these cattle were genotyped using the Illumina 50K v3 SNP chip. Breeding values for milk, protein, and fat yields, as well as protein and fat percentages were estimated using pedigree-based best linear unbiased prediction (PBLUP) and single-step genomic BLUP (ssGBLUP). Prediction accuracy was assessed as the mean of individual accuracies calculated from the prediction error variance, by excluding phenotypic records from two groups of animals consisting of the youngest genotyped individuals. The prediction accuracy for PBLUP ranged from 0.28 to 0.30 for milk yield, 0.26 to 0.29 for protein yield, and 0.27 to 0.29 for fat yield, whereas protein percentage ranged from 0.33 to 0.35 and fat percentage from 0.34 to 0.37. For ssGBLUP, accuracy for milk yield ranged from 0.58 to 0.61, for protein yield from 0.56 to 0.60, and for fat yield from 0.57 to 0.61. Furthermore, protein percentage ranged from 0.65 to 0.68, and fat percentage from 0.68 to 0.70. In conclusion, the inclusion of genomic information improves the accuracy of GEBVs relative to the use of pedigree information alone. Consequently, the size of the validation population strongly influences prediction accuracy, highlighting the need for larger genotyped reference populations in South African Holstein cattle.

Comparison of genomic evaluation models on simulated crossbred dairy cattle populations

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The use of crossbreeding in dairy cattle has gradually increased, reflecting breeders' interest in adopting new strategies to address current challenges in the dairy industry. However, the development of genomic predictions for crossbred populations was hindered due to limited data availability and the lack of adapted methodological tools. In France, the TransEvagenoc project, funded by APIS-GENE, aimed at developing genomic evaluation methods for crossbred dairy cattle. The objective of this study was to evaluate, using simulation, the relative performance of four genomic evaluation strategies in crossbred dairy cattle, to provide guidance on their implementation according to the available phenotypic data in crossbreds. Four methodological options were considered: (i) applying the ssGBLUP method as developed for pure breeds assuming the same SNP effects across breeds of origin, (ii) applying SNP effects estimated in each pure breed to crossbred genotypes using breed-of-origin of alleles information, (iii) adapting purebred SNP effects by incorporating crossbred information, and (iv) estimating SNP effects directly from crossbred genotypes and performances. The accuracy of models (iii) and (iv) was assessed regarding the number of crossbred information. Three simulated populations representing dairy breeds were generated using the AlphaSimR package. Five traits were simulated with genetic parameters resembling milk yield, fat yield, protein yield, somatic cell score, and interval calving to first service. Each breed underwent ten generations of selection with different trait weightings to create breed-specific trait strengths and linkage disequilibrium patterns. The simulated genome consisted of six chromosomes with 10,000 segregating sites, and 500 QTL with effects drawn from a Poisson distribution. Purebred lines were maintained under selection for ten additional generations, and a subset of purebred animals was used to supply a three-breed rotational crossbreeding system. In each generation, new F1 females were produced and introduced into the rotation, whereas crossbred females were mated to sires from a different pure breed in a rotational sequence. This design generated a continuum of crossbred individuals with varying breed contributions over ten generations. Population sizes were defined according to different scenarios, based on the targeted number of crossbred individuals made available for the genomic evaluation. Realistic genotypes and phenotypes were simulated for individuals from generations 10 to 20 and used to perform genomic evaluations. For each method, genomic prediction accuracy, i.e. correlation between true and estimated breeding values, were evaluated across increasing crossbred reference population sizes. Accuracy curves were compared to identify the most efficient strategy under different data availability scenarios. The results provide practical guidance for breeding organizations regarding the level of crossbred genotyping effort required to justify the implementation of the more advanced genomic evaluation methodologies.

Unknown Parent Groups in Turkish Holsteins: Metafounders Approach

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In the genomics era, the management of missing parental information can present significant challenges, particularly in cases involving so-called 'phantom parents' (Unknown Parent Groups; UPGs). The metafounders (MF) approach was developed, seeing UPGs as a group of interconnected bases and recognising that there could be more than one. If there are multiple relationships, the definition of relationships is done using a Gamma matrix (Γ), where diagonal elements correspond to self-relationships and off-diagonal elements amongst them. The objective of this study was to evaluate the effect of different MF grouping strategies on genomic breeding value predictions for lactation milk yield and calving interval in Holstein cattle. In the present report, a total of 11055 Holstein genotyped with 71639 variants. The pedigrees contain 234162 animals, of which 93601 and 53831 have observations of first 305-day lactation milk yield (LMY) and first calving interval (CI) records, respectively. To eliminate missing parent effects, two different grouping strategies were tested. Firstly (S1), missing parents were identified, using "Bpop", as belonging to six bases based on their origins (TR, US and others), and sex. The second strategy (S2) involved combining the previous definitions and separating the groups by considering different time spans (n = 17). The models were selected as ABLUP, ssGBLUP and ssGBLUP-MF with respect to univariate and bivariate models, conducted with "BLUPF90+". For MF models, genetic variance-covariance components were scaled, and predictions of each model were considered with consistency (ρ), bias, dispersion (β_1) and accuracy (acc) to evaluate MF effects with the focal group (born in 2018 – 2022 time span, both genotyped and phenotyped cows). The inbreeding levels of defined MFs range between 0.455 and 0.498 for Γs_1 and 0.455 to 1.997 Γs2, respectively. Among relationships of groups were observed as 0.400 – 0.460 range for Γs_1 and -0.447 – 0.904 range for Γs2, respectively. In addition, the validation results showed that ssGBLUP models provided more consistent and accurate breeding value predictions than the ABLUP model for LMY. The minimum ρ and acc were 0.86 and 0.52, respectively, for ssGBLUP models and 0.79 and 0.46, respectively, for the ABLUP model. All models exhibited slight bias (maximum of 80 kg and 0.14 days for LMY and CI, respectively) and some dispersion ($0.88 \leq \beta_1 \leq 1.00$). Based on these findings, ssGBLUP, particularly when combined with the MF approach, is recommended for routine evaluation of Holstein cattle populations affected by missing parental information. In particular, the grouping of unknown parents by both origin, sex, and time period may better capture differences among base populations, although the wider range of relationships observed in the more detailed grouping strategy suggests that careful definition of MF groups is essential. Further work is needed to optimise the definition of MF groups and to assess their usefulness for other economically important traits.

Combining sustainable poultry farming and meat quality: Insights from the intaqt project

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Chicken production and consumption today occupy a central role in global food systems. Poultry has become the most produced and consumed meat, with chicken representing nearly 90% of this production. The sector largely relies on intensive systems using fast-growing strains, which offer high technical and economic efficiency and allow the production of large volumes at low cost. However, in Europe, societal expectations regarding animal welfare, product quality, transparency, and sustainability are driving an increasing diversification of farming systems, with the development of more extensive models, lower stocking densities, and slower-growing strains. In this context, the European Horizon 2020 project INTAQT aims to better understand the relationships between farming practices and the intrinsic qualities of animal products, particularly chicken meat. The project follows an integrated approach combining the characterization of farming systems, multi-criteria evaluation of product quality (nutritional, sensory, technological, and safety aspects), and the development of authentication and traceability tools. The project is based on a network of 90 farms across five European countries (France, Italy, Belgium, Switzerland, and Poland), covering a broad continuum of systems, from the most extensive to the most intensive. The results show that the farming system strongly influences several dimensions of chicken meat quality. Intensification, notably through the use of fast-growing strains such as Ross 308, increases breast yield but also alters meat composition, with higher lipid content and lower protein levels. Concentrations of bioactive dipeptides, such as anserine and carnosine, recognized for their antioxidant properties and potential benefits for human health, also decrease as animal growth rate increases. Intensification also affects certain technological and sensory properties of the meat. Breast fillets from intensive systems generally exhibit higher water losses both raw and cooked, which can impact product appearance and processing suitability. Furthermore, the frequency and severity of muscular defects, such as white striping, wooden breast, or spaghetti meat, increase in systems using fast-growing strains, particularly for animals slaughtered at higher weights. Conversely, systems with more moderate growth rates appear to maintain good nutritional, technological, and sensory quality. The results indicate that an average daily gain below 50 g/day offers a favorable compromise between production performance and product quality. More broadly, the INTAQT project highlights the interactions between product quality, environmental sustainability, and animal welfare. It provides valuable insights to guide the evolution of production systems toward models that balance meat quality, sustainability, and economic competitiveness, in line with the "One Quality" concept.

Nutritional outcomes of high-intensity broiler farming with regard to amino acid patterns and protein digestion efficiency

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Meat is widely recognized as a valuable source of high-quality protein and essential amino acids, unlike most plant-based foods, which require combination to achieve a complete amino acid profile. Among animal products, broiler chicken breast is particularly rich in protein. However, the genetic selection for fast growth in modern broiler lines has led to the emergence of muscle abnormalities such as wooden breast, white striping, and spaghetti meat, which are associated with deteriorated meat sensory, technological and nutritional quality. Within the framework of the H2020 INTAQT project, we assessed the quality of chicken breast meat from 32 farms representative of six poultry production systems in Europe. These systems vary by growth rate, slaughter age and weight, and access to outdoor areas: Indoor_Fast_Heavy, Indoor_Fast, Outdoor_Fast_Heavy, Outdoor_Medium, and Outdoor_Slow—the latter including Label Rouge and male layer hybrids. In addition to standard sensory, technological, nutritional, and sanitary assessments, our study focused on protein digestibility and amino acid composition. Breast meat samples were minced, cooked to 70 °C core temperature, and subjected to in vitro digestion. Protein content was measured before and after digestion; small and large peptides were quantified via the Kjeldahl method, and free amino acids were analyzed using HPLC. The lowest protein content and digestibility were found in meat from the Indoor_Fast_Heavy system, characterized by the highest daily weight gain (>60 g/day) and slaughter weights of 2.6–2.8 kg. Essential amino acid content was higher in meat from broiler raised in systems Outdoor_Medium (56 days) and up to 80 days than those raised in system Indoor_Fast (35 days). Leucine levels, crucial for muscle protein synthesis, were significantly higher in all systems compared to Indoor_Fast_Heavy. Arginine, histidine, and tryptophan release during digestion was also consistently lower in samples from the most intensive system. Whatever the farming practices, the sum of essential amino acids meat containing did not differ. But for specific EAA, slight differences are noticed in particular for tryptophan and histidine, which is in line with the reduced availability observed after digestion. These findings underscore the significant influence of production systems on the nutritional quality of chicken meat, particularly in terms of amino acid profiles, and raise concerns about the impact of intensive farming practices based on fast-growing genotypes. The dataset will be further enriched with complementary quality indicators, including the incidence of breast muscle myopathies, to investigate potential links between meat nutritional value and the prevalence of these muscle disorders.

Effect of outdoor enrichment and dietary supplementation with n-3 fatty acid precursors and antioxidants on meat quality of free-range chickens

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Growing consumer interest in animal welfare and product quality is increasing demand for products from less intensive farming systems. In poultry, dietary strategies such as supplementation with n-3 fatty acid precursors can enhance meat nutritional value. In outdoor systems, enriched pastures (e.g., grass and trees) promote locomotor activity and grass intake, facilitating the transfer of bioactive compounds into the meat. However, increased activity and higher n-3 PUFA levels may enhance oxidative processes, potentially affecting meat quality, particularly in thigh muscles, which are lipid-rich and highly involved in movement. The aim of this study was to assess the combined effects of outdoor enrichments and dietary supplementation with n-3 PUFA and antioxidants on the quality attributes and oxidative stability of thigh meat from free-range reared chickens. Four commercial chicken farms using males of a slower-growing genotype were considered in the study: two farms showing the outdoor area without pasture (NP), and two with the outdoor area enriched with grass and trees (GT). On each farm, two pens (20,000 birds each) were assigned to either a control (C) or a supplemented diet (S; 5% linseed, vitamin E and selenium). Birds were slaughtered at 56 d of age. Thigh samples were collected to assess the main meat quality traits (i.e., pHu and colour) (n=120; 15/group) and oxidative status (TBARS and carbonyl content; n=64; 8/group) after 0, 3, and 7 d of refrigerated storage (4°C). Data were analyzed by two-way ANOVA, including the outdoor enrichment and the diet, as well as their interaction in the model. The differences observed on fresh meat (0 d of storage) indicated that the outdoor enrichment have played a more prominent effect than the dietary treatment on meat characteristics. In particular, higher pHu as well as lower lightness were observed in GT samples compared to NP (6.11 vs. 5.92; $p<0.001$ and 53.6 vs. 54.8; $p<0.001$, respectively). During refrigerated storage, oxidative processes affected both the lipid and the protein fractions, regardless of pasture type and diet. In detail, the highest TBARS values observed in the GT-S group at d 0 (1.70 mg MDA/kg meat; $p<0.05$) may be attributed to the combined effect of increased locomotor activity in the birds belonging to this group that, concurrently, had higher n-3 PUFA levels in the meat (data not shown). In fact, long-chain n-3 PUFA are very susceptible to peroxidation due to their chemical structure. In the present study, GT-C group showed the highest level of carbonyls at 3 d of storage (6.69 nmol/mg of protein; $p<0.05$). This can be mainly ascribed to the high locomotor activity of birds fed the C diet, which did not receive antioxidants supplementation. In fact, it is likely that the antioxidant content of the C diet was not sufficient to mitigate the oxidation process triggered by the increased movement. In conclusion, dietary enrichment with n-3 PUFA and antioxidants can differently affect chicken meat quality depending on the presence of grass and trees, which may enhance animal movement and promote muscle oxidative processes. This study is part of the INTAQT Horizon 2020 project, which aims to establish a link between husbandry systems and the intrinsic quality of animal products.

Identifying farming practices associated with beef fatty acids, trace minerals and antioxidants across European production systems

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This study investigated the relationship between combinations of farming practices and variation in multiple classes of nutritional traits in beef, including fatty acids (FA), trace minerals (TM), and antioxidant compounds (AO), across diverse European production systems under commercial conditions. During two slaughter periods, samples of the Longissimus lumborum muscle were collected from 489 animals originating from 53 farms across five European countries, representing a broad production-intensity gradient. The samples were pooled by farm and slaughter period, resulting in 99 observations. Farm data were obtained via structured questionnaires and comprised 33 features describing diet, herd composition, management, and land use. A machine-learning framework combining optimized Random Forest models and Conditional Inference Trees was used to identify farming features associated with variation in beef nutritional traits. The composition of polyunsaturated FA (PUFA) showed high predictability and clear system-level patterns: n-3 PUFA were associated with grass-based diets, dual-purpose breeds, and longer finishing durations, consistent with low-input agroecological production contexts. Conversely, n-6 PUFA were associated with high concentrate use and short indoor finishing periods, which is consistent with high-input, intensive production contexts. Predictability was lower for saturated and monounsaturated FA. TM and AO traits differed in their responsiveness to farming practices. Higher concentrations of iron and heme iron were associated with heavier slaughter weights and reflected a muscle oxidative metabolic type at slaughter, which was found in low-input, slow-growing systems. In contrast, Se and Zn exhibited low predictability. The content of the bioactive dipeptide compound carnosine was associated with farming practices reflecting breed composition and finishing strategy, with the highest concentrations found in low-input fattening systems containing higher proportions of dual-purpose breeds. Anserine and α-tocopherol exhibited lower and more variable predictability. Overall, FA traits were primarily associated with feeding-related lipid metabolism, as expected. AO and TM traits reflected broader muscle metabolic characteristics associated with the structure of the production system. Traits with weaker system-level predictability likely reflect physiological homeostasis or mechanisms not captured by the recorded farm-level practices. This highlights the need to interpret system-level effects through trait-specific biological mechanisms, rather than assuming uniform responses across production system categories.

Occurrence of persistent organic pollutants, mycotoxins and heavy metals in liver of beef in different farming systems

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The occurrence of contaminants in beef products can be related to cattle feeding, which generally differs among farming systems. Important contaminants in beef liver are polychlorinated dibenzo-p-dioxins (PCDDs) and dibenzofurans (PCDFs), dioxin-like polychlorinated biphenyls (PCBs), perfluorinated alkyl substances (PFAS), heavy metals and mycotoxins. These compounds were investigated in 550 beef liver samples from different farming systems collected from 55 European farms. The farms were categorized in five groups according to the age of the cattle, grazing and the proportion of concentrate feed and maize silage in the diet: Group 1 consists of grazing farms that fatten culled suckler cows (SuC) slaughtered at 90 months. Groups 2 and 3 are fattening young animals with grass-based diets and no or low concentrate feeds slaughtered at 18 (Grass18) and 28 (Grass28) months, respectively. Groups 4 and 5 are young animals (slaughtered at 15 months) that are fattened indoor with diets including medium (IndM) or high proportion (IndH) of concentrates and maize silage, respectively. None of the analysed contaminants exceeded the official maximum limits. The sum of PCDDs/PCDFs and dl-PCBs (expressed as WHO toxicity equivalent using the upperbound concentrations calculated with WHO toxicity equivalence factors 2005) showed significantly higher values for Grass18 (0.12 and 0.79 ng/kg liver, respectively) compared to IndH (PCDDs/PCDFs, 0.04 ng/kg liver) and IndM (dl-PCBs, 0.02 ng/kg liver). From 23 analysed PFAS, only Perfluorodecanoic acid, Perfluoroundecanoic acid (PFUnDA), Perfluororooctanesulfonic acid (PFOS) and Perfluorononanesulfonic acid were detected slightly above the limit of quantification. Significant differences were only found for PFOS and PFUnDA with the highest values for Grass18 (1.21 and 0.06 µg/kg liver, respectively). All of the 31 analysed mycotoxins were detected in low concentrations (<2.1 µg/kg liver) with no clear pattern among treatments. Equally, no clear pattern was found for heavy metals: for Cr there were no significant differences (mean of 20.1 µg/kg liver), for Ni and Pb, the highest values were found in Grass28 (24.7 and 12.8 µg/kg liver, respectively), for As in Grass18 (8.6 µg/kg liver) and for Cd in SuC (79.2 µg/kg liver). Overall, the highest values for the minimal amounts of persistent organic pollutants were found in farms with intensive grassland management and a low input of feed from arable land. Lower values were found in farms with suckler cows, with access to pasture and an extensive grassland management. Therefore, it can be concluded that access to pasture or more extensive green forage–based feeding do not result in an increase in food safety–relevant parameters in meat – at least in areas without known contamination with the above-mentioned contaminants. The study is part of the INTAQT project (funded by EU H2020 101000250).

Linking dairy system, breed, and welfare indicators to infrared-predicted fine milk composition and cheese-making traits in Parmigiano Reggiano cheese production area

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Milk technological traits result from the interaction among dairy system, genetics, and cow welfare, factors that also shape public perception of product quality and sustainability. Fourier Transform Mid-Infrared (MIR) spectroscopy is a valid and rapid tool for monitoring milk composition and predicting technological traits, yet few studies have evaluated the combined effects of dairy systems and animal welfare (AW) within a single production system. This study investigated the effects of dairy system, breed, and AW scores based on the Italian official AW assessment (CReNBA) on fine milk composition and cheese-making traits predicted by MIR spectroscopy within the Parmigiano Reggiano production area. Data included 2,175,148 test-day records, 1,869,687 MIR spectra, and farm-level and CReNBA data from 983 farms. Clustering based on feeding and housing systems, geographical area, breed (Holstein Friesian – HF, dairy Crossbred – Cr, Brown Swiss – BS, Simmental – Si, Reggiana – Re), milk yield, and herd size identified 4 dairy systems: traditional Apennines (TA), traditional Po Plain (TP), modern without (MWTMR) or with (MTMR) total mixed ration. CReNBA indicators included management (MGT), structure and equipment (SAE), and animal-based measures (ABM), and were classified into low, medium, and high scores according to compliance with AW standards. The predicted traits, i.e. protein and macromineral profiles and cheese-making traits, were obtained using gradient boosting machine learning method developed on an external calibration population (R^2 from 0.58 to 0.88) and analyzed using mixed models with fixed effects of dairy system, breed, AW class, lactation stage, parity, month, and year, and random effects of animal and farm. Breed, dairy system, and their interaction resulted significant for almost all traits, suggesting specific breed attributes and a production context-dependent expression of breed potential. Milk yield increased from traditional to modern systems for all breeds, except Re, which performed better in TP farms. Fat content showed the opposite trend to milk yield, while protein and casein concentrations increased toward modern systems, with breed-specific proportions. Somatic cell count (SCC) was lower in TA farms, but Si showed lower values in TP and Re in MTMR. BS had higher fat, protein, and urea content. Indeed, Re and Si showed higher true protein and casein, with Re performing better in traditional systems and Si in modern ones. Minerals declined to varying extents from traditional to modern systems. Cheese-making traits were improved in modern systems with higher cheese yields and more standardized coagulation, although Si showed better performance in TA. Regarding AW, milk yield was more affected than milk composition, increasing with higher MGT and ABM scores. Higher scores in different areas were associated with higher lactose content and casein index, lower SCC, reduced urea, lower αS2-CN and α-LA, increased mineral content, improved cheese yield (mainly due to greater water retention), and slightly faster coagulation with firmer curd. Overall, breed performance was strongly linked to production context. Albeit to a limited extent, AW influenced fine milk composition and technological traits, confirming the value of routine MIR-based assessments for monitoring dairy herd performance. This research was conducted within the INTAQT project funded by the EU Horizon 2020 programme (grant No. 101000250).

A harmonized methodological framework for multi-criteria assessment of intrinsic quality in EU livestock products

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Ensuring high-quality livestock products that comply with EU regulations while meeting both industry requirements and consumer expectations remains a major challenge for the livestock supply chain. The EU INTAQT project investigates how farming system intensification affects the potential intrinsic quality of milk, beef, and chicken taking these different expectations into account. This study aimed to develop three harmonized multicriteria assessments of the potential intrinsic quality of milk and meat at the farm gate for UHT milk, beef striploin, and chicken breast final products. A common methodological framework was first established by a scientific working group representing the three product sectors. Four principles, describing specific dimensions of intrinsic quality (nutritional, health, sensory, and technological) were defined, each subdivided into criteria. Conciliations between experts were then conducted to define the structure of the hierarchical assessment tree (indicators → criteria → principles) and the assessment (scoring of indicators, weighting). Theses conciliations were performed separately for each product, except for the safety indicators, which were addressed by a single cross-product expert group. Each conciliation followed a three-step process: (1) Individual elicitation, during which experts selected indicators from the list of indicators assessed in the INTAQT project to define the hierarchical assessment tree, for example; (2) Collective discussion in a three-hour online meeting to synthesize proposals and reach consensus; and (3) Validation, allowing experts two weeks to confirm or revise the written summary. An initial series of expert conciliations was organised to define the indicators characterising each criterion. For the UHT milk, two meetings were required to finalize indicators for the nutritional and health principles, resulting in 3 nutritional and 12 health indicators. A single meeting addressed the sensory and technological principles, yielding 11 sensory and 4 technological indicators. The joint meeting on safety aspects specifically identified 13 safety indicators. Equivalent procedures were implemented for beef striploin and chicken breast. A second round of expert meetings focused on defining indicator thresholds to convert raw measurements into standardized scores (1–10) based on INTAQT project databases, and on establishing the weightings used to aggregate indicators into criteria and criteria into principles. This phase required at least two meetings per product for the nutritional & health dimensions and for the sensory & technological dimensions, as well as a joint meeting for safety-related aspects. In a final step, European stakeholder groups determined the weightings of the four overarching principles for each product, through three separate meetings per product held in different countries. These multicriteria assessments will be applied to data collected through an extensive survey and sampling campaign conducted on European farms representing a gradient of intensification levels for each product. This approach will enable evaluation of how farming system intensification affects the potential intrinsic quality across the three livestock products. The study is part of the INTAQT project funded by the EU's H2020 research and innovation programme (grant agreement N°101000250).

Number of animal lives vs. quality of life in broiler, dairy, and beef production

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Animal welfare is often defined as animals' quality of life, which depends on their living conditions. Conditions of death also matter, as slaughter is often accompanied by suffering, but death per se is generally not considered a welfare issue. However, animals may have interests in continued life. From an animal perspective, one should thus consider both the quality of animals' life and the number of animal lives required to produce animal products. We questioned whether it is possible to maximise the first while minimising the second, across or within livestock productions. We used data from 18 broiler, 15 dairy, and 3 suckler systems (~5 farms/system) in Europe (Horizon2020 INTAQT project). We calculated the number of lives per kg of protein from meat and milk. We approximated quality of life from the averaged percentage of time spent outdoors per life (positive experiences) and health disorders within each production (negative experiences). For cattle, cows and offspring were considered, with calves fattened in confined or pasture-based systems. Across productions, dairy production required on average 5 times fewer lives than suckler systems and over 1,000 times fewer lives than broiler systems to produce the same amount of protein (4 ± 2, 22 ± 10 and 5,000 ± 1,500 lives/t protein in dairy, suckler, and broiler systems). Suckler systems provided the highest outdoor access per life (43 ± 6 vs. 13 ± 14 and 12 ± 13% in dairy and broiler systems). In broilers, moving from standard to heavy production reduced the number of animal lives by 25-90%, but could increase morbidity. Moving from standard to outdoor systems using slow growing broilers slaughtered at a heavier live weight reduced the number of animal lives by 4-16% and improved their quality of life. However, a broiler system combining the fewest animal lives and the greatest amount of outdoor access (Ross308 genotype in an organic system) had numerous health issues such as lameness and leg deformations. In cattle, a trade-off was highlighted: the most productive dairy systems required the fewest animal lives but with low outdoor access (2.5 ± 0.3 lives/t protein, 5 ± 5% outdoor access per life with 15 ± 17% for cows and 0% for calves), whereas the most extensive dairy systems and suckler systems provided the greatest amount of outdoor access but required the largest number of animal lives (for the most extensive dairy systems: 4.9 ± 0.5 lives/t protein, 24 ± 16% outdoor access per life with 59 ± 14% for cows and 15 ± 19% for calves; for suckler systems: 43 ± 6 % outdoor access per life, with 54 ± 12 % for cows and 40 ± 7% for calves). Grass-based fattening of calves improved the average life quality and could also reduce the number of animal lives if slaughter weight is increased. Within production, minimising the number of animal lives while maximising their quality of life is not always possible, and the question of how to weigh the two objectives remains open. Both objectives can be improved by fattening animals outdoors to a higher slaughter weight in broiler and cattle but this can lead to higher morbidity in broilers if fast-growing genotypes are used. Across productions, dairy production best minimises the number of animal lives with some systems maximising quality of life. Reducing the number of animal lives while improving their quality of life would thus require a change in human diets to favour meat from dairy production and, within each livestock production, products from alternative systems that achieve higher slaughter weights while providing good living conditions to all animal categories.

Milk mid-infrared spectra potentially authenticates farming system intensification level and season of production

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Food fraud is a persistent challenge in global supply chains, and authentication systems are necessary to reassure buyers that claims such as “organic,” “animal-friendly,” or “origin-certified” are truthful. This study assesses the potential of using Fourier-transform mid-infrared (FT-MIR) spectra for authentication purposes regarding farming intensification level and season of production. A total of 65 dairy farms in France, Germany, Switzerland, and Ireland were visited collecting a total of 361 samples during summer (66%) and winter (34%). Farming intensification level were categorized in 5 classes through a hierarchical clustering based on feed inputs, farm animal density, pasture stocking rate, and fertilization levels. The farm classes range from low feed input using low amount of concentrate and mainly grass from land managed extensively (17% of the farms) or intentively (21%) to farms with high feed input from concentrates and maize silage with low (32%) or high (18%) stocking rate on pastureMilk spectra from each country were standardized to build discriminant models using Principal Component Analysis (PCA) and Partial Least Squares-Discriminant Analysis (PLS-DA) for farming practices intensification and season. For the PLS-DA, the dataset was split into 70:30 (training set:testing set). Models’ performance was evaluated via balanced accuracy. Results revealed that the PCA explained 82% of the variance (PC1, 65%; PC2, 17%) but showed no apparent clustering by season or intensification level, indicating that this unsupervised method was insufficient for sample authentication. Better results were obtained with the PLS-DA, as this supervised method was able to discriminate samples. In particular, excellent classification was observed for the season, with a high accuracy of the model (testing set balanced accuracy, 0.91) confirming that MIR spectra capture compositional changes linked to seasonal feeding strategies, such as pasture-based diets in summer versus conserved forage in winter. However, prediction performance for farm intensification level varied widely, with the best results for discriminating extensive farms (testing set balanced accuracy, 0.78), while most intensive farms revealed the lowest discriminatory potential (testing set balanced accuracy, 0.52). This lack of specificity suggests that MIR detects diet-related changes but cannot fully capture nuances of management, such as partial grazing or mixed feeding strategies. In conclusion, FT-MIR spectroscopy can effectively authenticate the season milk was produced, which could be a proxy for fresh grass consumption. However, further refinement and larger datasets are needed to improve the classification based on farming intensification.

Infrared spectroscopy for farming system classification and beef quality assessment: a multi device chemometric approach

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One of the aims of the EU INTAQT project was to develop innovative and cost-efficient analytical tools for the rapid prediction of intrinsic quality traits and the authentication of husbandry systems across Europe. We evaluated and compared the performance of two portable spectroscopic devices, the IndiGo Vis (visible region, 400-700 nm) and the FlameNIR (NIR region, 935-1665 nm), with that of a benchtop reference instrument (NIRS™ DS2500, combined Vis/NIR region) for authentication and quality assessment in the beef production chain. The first objective of this study was to classify beef samples according to the intensification level of the farming system, previously defined based on farming input data. For this purpose, we used a linear discriminant analysis with previous variable selection algorithm based on covariance among wavelengths. The IndiGo Vis and FlameNIR devices showed classification abilities comparable to the NIRS™ DS2500, achieving testing accuracies of 0.72-0.78 when considering the farm as the experimental unit and 0.82-0.90 when considering the animal as the experimental unit. The second objective was to predict a wide range of beef biochemical quality attributes, including proximate composition, fatty acids profile, oxidation products, B-group vitamins (and others), using partial least squares regression. In this context, the NIRS™ DS2500 achieved the highest overall performance (R^2 = 0.65-0.93, depending on the trait), whereas the portable devices generally produced lower prediction accuracy. Among the latter, only the IndiGo Vis yielded reliable prediction models for total and heme iron (R^2 = 0.69 and 0.77, respectively), likely because these traits are linked to fresh meat colour and therefore detectable within the visible spectral region. As part of this objective, we also compared spectral data collected on pooled samples (Pooled) with those obtained on individual samples and subsequently averaged (Ind_Aver). Prediction accuracy differed significantly between the two strategies ($p<0.001$), with consistently higher R^2 values for Ind_Aver (0.65) compared to Pooled (0.22) across all traits. The third objective was to predict beef carcass characteristics measured at the slaughterhouse, such as pH and marbling score, plus meat tenderness (shear force) and sensory attributes. Although the NIRS™ DS2500 again outperformed the portable devices, the resulting prediction equations were unsatisfactory ($R^2 < 0.30$), regardless of the instrument used. However, some classification approaches might provide more robust results than exact value prediction (analysis ongoing). The studies showed that the tested portable devices could support specific applications for authentication and for prediction of some biochemical traits, offering promising perspectives for practical field use. Nevertheless, developing robust calibrations for a broader set of quality traits remains challenging and further methodological refinement will be necessary to improve prediction accuracy and expand the applicability of portable spectroscopic tools.

Evaluating the Impact of Traditional Montanera vs. Intensive Feeding Systems on Technological Meat Properties in Iberian Pigs: Implications for System Resilience

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Climate-driven declines in acorn availability during Montanera compromise the resilience of the traditional Iberian pig system, forcing producers to adjust stocking rates and increasingly rely on standard compound feed with no acorn inclusion. This dietary shift may modify essential meat-quality attributes, influencing texture parameters such as hardness, adhesiveness, springiness, cohesiveness, gumminess, chewiness and resilience, together with physicochemical traits including dry matter, fresh protein, total fat, water-holding capacity (WHC)and pH. These changes pose challenges to maintaining the technological and sensory characteristics historically linked to acorn-based finishing. Evaluating how standard feed affects these indicators is essential for designing adaptive strategies that enhance robustness in European livestock systems facing continuous environmental variability. A total of 40 purebred 100% Iberian Retinto pigs were selected for the trial. Ten of them were fed under traditional Montanera conditions (extensive free-range system, stocking density 0.75 pigs ha^{-1}) with exclusive access to naturally available acorns and pasture, until the end of the finishing phase, while another ten were assigned to an intensive feeding regime, although maintained under extensive conditions with a stocking density of 100 m² per animal and fed a high oleic commercial compound feed. Both production systems were carried out in accordance with the Iberian Quality Standard (RD 4/2014). After slaughter, the longissimus thoracis et lumborum (LTL)-loin– and Serratus ventralis -presa– muscles were removed from the carcasses and analyzed fresh. Different quality parameters were evaluated, including the texture profile at 80% compression, WHC, and proximate analysis. The results revealed significant differences between production systems across several quality parameters. Compared with Montanera, pigs raised under intensive conditions and fed a high-oleic commercial diet exhibited significantly higher ($p < 0.05$) maximum force and hardness (N; ~30 points higher), at least 10-point increases in gumminess and chewiness, and lower WHC and higher moisture in both presa and loin cuts. Moreover, a strong correlation was observed among texture parameters, WHC, and moisture in both cuts, indicating a consistent relationship between structural integrity and water retention. Finally, a significant interaction effect between production system and muscle type was detected in the nutritional composition ($p<0,005$), suggesting that the response of each cut to the feeding regime differed in magnitude and direction. The production system significantly influences the technological properties of meat, particularly hardness and chewiness, whereas proximate composition parameters (moisture, dry matter, fat, and protein) are primarily determined by muscle type and its interaction with the production regime. WHC constitutes a structural mediator between composition and texture, indicating that the effect of the production system on mechanical properties is partially driven by modifications in muscle microstructure and water–protein interactions. This research was funded by the Regional Government of Extremadura and the European Regional Development Fund (FEDER), Project " IBERCAL " The registration and attendance have been co-financed—85%—by the E.U., through FEDER Fund, and by the Regional Government of Extremadura. GR24147.

Factors influencing the phospholipid profile and concentration in cow milk: A study in commercial European dairy farms varying in intensification level

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Phospholipids (PL) are amphiphilic lipids located in the membrane of milk fat globules and milk exosomes. Phospholipids can be grouped into 5 classes according to their structure: phosphatidylethanolamine (PE), phosphatidylcholine (PC), sphingomyelin (SM), phosphatidylserine (PS), and phosphatidylinositol (PI). A higher intake of PL may provide beneficial health effects for consumers by supporting brain development, promoting gut health, regulating plasma cholesterol, and reducing inflammation. Therefore, the PL profile and concentration can be considered as a health quality trait of milk. A limited number of previous studies have shown that some farming factors can influence the PL concentration in milk and milk fat (g/100 g of milk and g/100 g of total lipids, respectively). These factors include cow breed (Holstein vs. Jersey), lactation stage (early vs. late), diet (linseed supplementation, forage-to-concentrate ratio), season (summer vs. winter), and management (conventional vs. organic farming). Our objectives were: (1) to characterise the variability of milk PL concentration and profile according to farm intensification level in Europe, and (2) to identify factors influencing milk PL profile and concentration. Milk samples were collected in summer, on three occasions, from 80 commercial European dairy farms located in France, Germany, Ireland, Italy, and Switzerland. Milk samples were pooled per farm on a weight basis before freeze-drying. Phospholipids were extracted using the modified Folch method, separated using high-performance thin-layer chromatography, and quantified using densitometry. Results were expressed as % of total PL and as g/100 g of total lipids. Questionnaires completed at each farm visit were used to characterise farm intensification (Caumette, 2025) as well as management, including herd composition and diet, during the 7 days before each sampling. Results showed variability between farms in both PL profile (coefficient of variation ranging from 16.0 to 29.8%) and concentration (coefficient of variation ranging from 7.9 to 36.1%). However, the intensification level did not appear as a major factor in explaining the observed variability. Preliminary descriptive statistics highlighted a negative correlation between maize intake and total PL concentration ($r = -0.23$, $P = 0.047$). No evidence of correlation was found between the dietary phosphorus content and PL profile or concentration. Among major milk PL (PE, PC, and SM), fresh grass intake (grazing or zero-grazing) was positively correlated with PE ($r = +0.25$, $P = 0.031$) and SM ($r = +0.29$, $P = 0.012$) concentrations, whereas maize intake was negatively correlated with PC ($r = -0.23$, $P = 0.048$) and SM ($r = -0.35$, $P = 0.002$) concentrations. The dietary content of C18:3 n−3 was positively correlated with SM concentration ($r = +0.35$, $P = 0.002$), whereas the dietary contents of C18:2 n−6 and C18:1 were negatively correlated with SM concentration ($r = -0.27$, $P = 0.019$ and $r = -0.31$, $P = 0.005$, respectively). Higher milk fat yield was correlated with lower PC ($r = -0.32$, $P = 0.005$) and SM ($r = -0.35$, $P = 0.021$) concentrations. Milk somatic cell count was negatively correlated with PE concentration ($r = -0.25$, $P = 0.032$). Overall, the observed correlations were weak. Future work will use multivariate statistical analyses, including random forest models, to investigate factors influencing milk PL profile and concentration.

Early-life antimicrobial treatment have long-term effects on the fecal microbiota of dairy calves

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Early-life antimicrobial therapy is widely used in calf management to control gastrointestinal diseases, yet its long-term effects on the gut microbiota remain poorly understood. Host genetics and management practices may influence the microbiota response to these interventions. This study aimed to evaluate the long-term effects of early-life antimicrobial treatments on the fecal microbiota of calves. Twenty animals were selected from a cohort of 63 based on health records. Two breeds were included, Holstein (n=10) and Normande (n=10), with equal representation of calves that experienced diarrhea (n=5) and calves that were healthy throughout the experimental period (n=5). Samples were collected at birth, at 1 week, at 3 weeks, at weaning, at 2 weeks post-weaning, and at 8 months of age. A total of 120 fecal samples were sequenced using bacterial 16S rRNA gene amplicons. As most of the digestive disorders occurred within the first 6 months of age, the potential long-term effects of antimicrobial treatments were evaluated at 8 months of age, when all calves were clinically healthy. All calves were raised indoors, but the etiology and, therefore, the treatment strategies differed between breeds. Holstein calves that developed diarrhea received broad-spectrum antibiotics administered intramuscularly before weaning. In contrast, Normande calves were treated orally with coccidiostats after weaning. At 8 months of age, both alpha and beta diversity differed significantly between Holstein and Normande calves ($P<0.05$). Diversity also differed between calves with a history of digestive disorders vs healthy calves ($P<0.05$), and a significant interaction between breed and health status was detected ($P<0.05$). The abundance of most prevalent families, including Prevotellaceae, Lachnospiraceae, Oscillospiraceae and Ruminococcaceae, did not differ between breeds or health status. However, the families Atopobiaceae, Flavobacteriaceae, Eggerthellaceae, and Desulfovibrionaceae were more abundant in Normande calves compared with Holstein calves ($q<0.1$). Conversely, Nocardiaceae, Microbacteriaceae, Pirellulaceae, and Victivallaceae were more abundant in Holstein calves than in Normande calves. Calves that received antimicrobial treatments following diarrhea episodes showed increased relative abundances of several bacterial families, including Marinifilaceae, Victivallaceae, Eggerthellaceae, Bacteroidaceae, Peptostreptococcaceae, Christensenellaceae and Pirellulaceae, compared with non-treated calves, which were characterized by higher abundances of Fibrobacteraceae and Desulfovibrionaceae. These results suggest that calves showed breed-dependent fecal microbiota responses, and these differences interact with prior health status history and with antimicrobial treatments. These findings underscore that, despite calves differed in breed, management conditions, antimicrobial compounds, and the timing of treatment, a long-term effect on fecal microbiota composition was observed. This suggests that early-life therapeutic interventions can induce persistent microbial shifts that extend well beyond the treatment period. Further studies are needed to determine whether these microbiota shifts influence later animal performance in dairy cattle.

The effect of transitioning from low energy to high energy diets, and vice versa, on viruses and their hosts in the rumen fluid and faeces of Holstein dairy cows

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The rumen microbiome, which consists of various microorganisms, will change when the diet of the ruminant changes. High-energy or high-concentrate diets have been reported to have a lower bacterial diversity compared to low-energy or high-roughage diets. However, little is known about the effect of the change from one energy diet to another, and vice versa, on the prokaryotic and viral community. The aim of this study was to determine the effect of transitioning cows from a low energy diet to a high energy diet and vice versa on the viral community and their hosts. Twenty first lactation Holstein cows were divided into two groups: one group received a low energy diet and thereafter a high energy diet (LE~HE) while the other group received a high energy diet at first and then a low energy diet (HE~LE). The animal experiment and sampling was done in the collaborative research project WiMiQ (https://gepris.dfg.de/gepris/projekt/202989534). Rumen fluid and faecal samples were collected three times across 6 weeks and subjected to DNA extraction and shotgun metagenomic sequencing. In total, 427 metagenome assembled genomes (MAGs) were assembled, and 4112 viral operational taxonomic units (vOTUs) were identified. Alpha and beta diversity of each community were determined, and functional annotation was performed on the generated contigs. In the rumen fluid, little variation was found in the alpha diversity of the viral community when the animals transitioned from HE~LE. A significant decrease in alpha diversity was observed in the group where the animals transitioned from LE~HE, which was also observed in the prokaryotic community. The Beta diversity clustered according to energy shift in both the prokaryotic and viral communities. After the transition from LE~HE, the evenness of the viral community decreased indicating that some vOTUs became dominant. The majority of these vOTUs were associated with an unknown host, but 4 vOTUs were linked to uncultured members of the Succinivibrionaceae family. High concentrate diets favour Succinivibrionaceae, where it rapidly ferments starch to produce succinate among others. When considered together, this might indicate that a bloom of specific phages occurred when the animals transitioned from LE~HE, possibly driven by the presence of Succinivibrionaceae. In contrast, there was no significant change in the abundance of Succinivibrionaceae and its phages when the animals were transitioned from HE~LE. The changes in bacterial and viral compositions need to be elucidated further to improve our understanding of the host-phage interaction and the possible consequences for the animal.

Metaproteomic Insights into Energy-Driven Microbial and Protozoal Functional Shifts Across the Ruminant Gastrointestinal Tract

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Ruminants rely on a symbiotic rumen and intestinal microbiome including bacteria, archaea, fungi, and protozoa. Current functional and genomic knowledge remains biased toward prokaryotes, leaving eukaryotes insufficiently characterized. Because diet and dietary energy shifts are major drivers of microbial restructuring in the gastrointestinal tract (GIT), this study used spatially resolved metaproteomics to investigate how changes in dietary energy alter the microbiome composition and functional activity across the GIT in dairy cows. The animal experiment and sampling was done in the collaborative research project WiMiQ (https://gepris.dfg.de/gepris/projekt/202989534). A total of 48 mid-lactation Holstein dairy cows (178.2 ± 23.5 days in milk; primiparous and multiparous) were randomly assigned at week −1 to two dietary energy levels—high energy (HE; 11.45 MJ ME/kg DM) and low energy (LE; 10.85 MJ ME/kg DM). From week 1 onward, dietary energy levels were reciprocally exchanged between groups to induce a controlled dietary energy shift, while all cows were offered a common diet supplemented with fava beans and ad libitum access to a partial mixed ratio consisting of 66% maize silage, 32% grass silage and 2% barley straw. Diet formulation and nutrient supply followed GfE (2023) recommendations. The trial lasted 7 weeks. At week 7, 20 animals (n = 10 per energy group) were slaughtered under standard welfare regulations. Mucosal and digesta samples were collected along the GIT to enable spatially resolved metaproteomic analysis. Proteins were extracted, digested, and analysed by LC–MS/MS. The MGnify cow rumen database, supplemented with protozoal SAG-derived sequences, was used as the search database in MetaLabMAG, while the UniProt bovine database served as the host reference. The obtained data suggest that dietary energy level influenced microbiome composition including protozoal species across GIT compartments. Beta diversity of animal- and section-specific metaproteomic samples differed significantly between dietary treatments, revealing a clear separation of microbiomes under HE and LE diets, consistent with energy-driven restructuring. Differential abundance analyses highlighted compartment-specific genera contributing to these compositional differences. Functional profiling further indicated diet-related differences in pathway expression, suggesting that dietary energy modulates microbial and protozoal activity as well as community structure. These findings underscore the importance of integrating spatial metaproteomics to better understand energy-driven microbial dynamics in ruminants.

Plasma Extracellular Vesicle Proteomics Reveals Systemic Metabolic Adaptation to Dietary Energy Transition in Dairy Cows

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Extracellular vesicles (EV) are nanosized lipid bilayer-enclosed particles that transport proteins, lipids and nucleic acids between cells, coordinating intercellular communication. Because their molecular cargo reflects the metabolic and physiological state of their cell of origin, circulating EV may serve as indicators of systemic metabolic adaptation. Dietary energy transitions are known to influence rumen microbial composition and host metabolism; therefore, we hypothesized that altering the concentrate-to-roughage ratio would modulate the plasma EV proteomics profile in dairy cows. The animal experiment and sampling were done in the collaborative research project WiMiQ (https://gepris.dfg.de/gepris/projekt/202989534). Twenty primiparous German Holstein cows (182 ± 15 days in milk) were assigned to either a high-energy diet (HE; 45% concentrate, n = 10) or a low-energy diet (LE; 25% concentrate, n = 10). At Week 1, diets were switched: HE cows received a low-energy diet (20% concentrate; HE/LE), and LE cows received a high-energy diet (50% concentrate; LE/HE). Plasma samples were collected before the transition (TP-1) and after six weeks (TP6). EV were isolated by differential centrifugation followed by size exclusion chromatography. Particle concentration and size distribution were measured by nanoparticle tracking analysis. EV-associated proteins were identified by LC–MS/MS (Orbitrap Fusion Lumos) and processed against the Bos taurus UniProt database at 5% FDR. Statistical analysis in R (v4.4) included variance stabilization, missing value imputation, and differential abundance testing using Limma. Functional enrichment analyses were performed using STRING (v12), g:Profiler, and ClueGO (v2.5.10). Plasma EV particle number and size distribution did not differ between dietary groups or time points. Proteomic profiling identified 404 plasma EV-associated proteins, 27 of which overlapped with the top 100 Vesiclepedia EV proteins. STRING analysis revealed a highly enriched protein–protein interaction network (PPI enrichment $p < 1.0 \times 10^{-16}$), indicating strong functional connectivity among EV-associated proteins. Differential abundance analysis showed that proteomic remodeling was primarily driven by time rather than dietary group, suggesting a coordinated systemic response to dietary transition rather than a passive temporal change. In the HE/LE group, 43 proteins were significantly regulated, while 27 proteins were differentially abundant in the LE/HE group at TP6 compared to TP-1. Enrichment analyses consistently identified pathways related to lipoprotein particle remodeling, complement activation, antioxidants, and endosomal trafficking. Although partial co-isolation of lipoproteins cannot be excluded, the strong network connectivity and consistent pathway-level enrichment support coordinated biological regulation rather than random contamination. In conclusion, dietary energy transition induced coordinated remodeling of the plasma EV proteome, reflecting dynamic systemic adaptation to altered energy supply. This study demonstrates that plasma EV proteomic profiling can provide novel insights into metabolic responses to dietary modulation in dairy cows.

Industry-level value and welfare consequences of methane-reducing feed additives in EU ruminant value chains

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Methane-mitigating feed additives are among the most promising near-term options to reduce greenhouse gas emissions from ruminant production, yet their deployment depends on incentives, feasibility across production systems, and how costs and benefits are shared along the value chain. This paper assesses the industry-level value consequences of alternative feed additives by linking farm-level effects to market and welfare outcomes "from farm to fork". We develop a scenario-based socio-economic framework that combines comparative static analysis with participatory modelling to quantify how additive adoption affects production costs, productivity, emissions intensity, prices, quantities, and surplus distribution across farmers, processors, retailers, and consumers. Empirically, we examine three additive pathways; 3-nitrooxypropanol (3-NOP), linseed oil, and extruded linseed; to quantify and compare their value-chain consequences under alternative uptake scenarios First, we parameterize additive-specific farm-level impacts (changes in unit costs, output, and emissions per unit of product) and upscale them using EU baseline market data. Second, we translate partial adoption into sector-level supply shifts and estimate resulting changes in market equilibrium under alternative assumptions on demand responsiveness and cost pass-through. Third, we extend the analysis beyond pure cost effects by allowing for demand-side mechanisms where verified low-methane products can attract price premia or secure market access. Scenario design is co-developed with value-chain stakeholders and structured as policy–market narratives that reflect plausible uptake pathways: (i) a reference regime with limited adoption driven by private profitability; (ii) incentive-supported regimes (e.g., eco-schemes, advisory support) that lower adoption barriers and broaden uptake; (iii) a high-regulation regime (e.g., carbon pricing or mandatory intensity targets) that accelerates adoption but may increase compliance costs and risk import substitution; and (iv) a coordinated value-chain regime based on contracts, certification/labeling, and premium-sharing arrangements that can shift incentives and redistribute value. The study delivers scenario-based estimates of (a) sector-wide emissions reductions, (b) changes in producer and consumer surplus, (c) distribution of gains and costs along the chain, and (d) the conditions under which mitigation becomes both scalable and economically robust. By comparing incentive-, regulation-, and market-pull pathways, the analysis provides actionable guidance on policy and contractual designs that support adoption while safeguarding competitiveness and fairness across ruminant value chains.

Enabling Methane Mitigation with Standardized Rumen Microbiome References and Pipelines

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Mitigating enteric methane is an urgent priority for sustainable cattle production. Assessing the level of mitigation achievable via changes in feed, microbiome modulation, and genetic selection depends on accurate, standardized rumen microbiome measurements, as methane is produced by methanogenic archaea from fermentation-derived substrates. Yet current bioinformatic practice limits precision and comparability: incomplete rumen microbiome references, modest strain-level resolution, functional annotation gaps, and sensitivity to wet-lab and Bioinformatics pipeline choices. Closing these gaps is critical for reliable microbiome–methane associations and scalable mitigation strategies. We addressed these challenges through three objectives: (1) benchmarking current sequencing technologies for taxonomic and functional quantification of the rumen microbiome; (2) developing a reproducible, modular pipeline for multi-purpose metagenomic analysis and construction of a non-redundant rumen gene catalog; and (3) curating high-quality rumen metagenome-assembled genomes (MAGs) into a versioned, queryable database. We generated 1232 Illumina short-read whole-metagenome sequencing (WMS) datasets, 52 Oxford Nanopore long-read WMS datasets, and 459 short-read 16S rRNA amplicon sequencing (16S) datasets, and publicly available datasets. We benchmarked platforms using metrics spanning community profiling accuracy, strain resolution, absolute quantification and functional annotation fidelity. To streamline analyses, we designed and released a modular pipeline covering read QC, assembly, polishing, binning/refinement/dereplication, taxonomy, and gene catalog construction. Finally, we present a curated rumen MAG database with standardized abundance estimates and taxonomic annotations to enable robust and comparable rumen microbiome analyses. In standard mock communities, both short-read and full-length 16S sequencing exhibited PCR-driven distortion and bias from inter-genome variation in 16S rRNA operon copy number, affecting quantitative accuracy. Full-length 16S nevertheless yielded more accurate abundance estimates. WMS approaches were limited in detecting and accurately quantifying low-abundance taxa. Across methods, we observed non-trivial rates of taxonomic misclassification—predominantly among closely related clades and under-represented lineages— which is expected to decrease with the use of curated, niche-specific reference resources. The consistent patterns were observed in ~450 samples profiled both by WMS and 16S. To put these findings into practice and support community-wide standardization, we developed MetaMAG Explorer (for database augmentation and multi-purpose metagenomic analysis) and MetaGene (for non-redundant gene-catalog construction and functional annotation). These pipelines yield a rumen-niche resource comprising 21667 non-redundant MAGs, a non-redundant gene catalog with standardized annotations, and harmonized 16S reference and annotation files, enabling reproducible abundance estimation for both WMS and 16S data. We benchmarked different sequencing strategy, mapped resolution–bias trade-offs, and released MetaMAG Explorer/MetaGene with curated rumen MAG, gene-catalog, and 16S resources to standardize rumen-relevant profiling. This study provides a robust measurement foundation for rumen microbiome studies and further accelerates the development of methane mitigation strategies for sustainable cattle production.

Response of primary mammary epithelial cells to pathogen challenge in dairy cows

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Mastitis is the major health and welfare challenge in dairy cattle, and a deeper understanding of the early molecular responses in the mammary gland is essential for developing sustainable prevention and breeding strategies. Using primary bovine mammary epithelial cells (pbMECs) derived non-invasively from milk, we characterized the genomic mechanisms underlying the early host response to Escherichia coli by in vitro challenging of pbMECs with E. coli. In addition, as new treatment options are needed to prevent further emergence of antimicrobial resistance, we tested the effect of lactic acid bacteria (LAB) on the host response during the pathogen challenge. Metabolites from LAB are reported as potential candidates as alternative therapeutic agents for bovine mastitis. To investigate how host genetics modulate functional responses, pbMECs from cows with divergent genomic breeding values for udder health were compared under identical E. coli challenge conditions. Transcriptome profiling at 3 h and 24 h post E. coli challenge revealed a rapid, robust activation of innate immune pathways, dominated by strong upregulation of chemokines, cytokines, and inflammatory signaling cascades, including TNF, NF-κB, NOD-like receptor, and cytokine–cytokine receptor interaction pathways. Antigen presentation pathways became prominent at later time points, indicating a progression toward adaptive immunity. When pbMECs were challenged simultaneously with LAB and E. coli, gene function of the host was adjusted compared to the challenge with E. coli only, as more genes were both up- and downregulated at early stages of challenge. On the other hand, when pbMECS were challenged with LAB alone, only very few host genes were differentially expressed. Cells from cows with lower genomic breeding values for udder health displayed markedly higher numbers of differentially expressed genes and stronger modulation of metabolic and cell-cycle pathways, whereas cows with higher breeding values for udder health showed a more focused and regulated inflammatory response. Despite these extensive transcriptional differences, early epithelial responses did not overlap known mastitis associated genomic regions, suggesting that the initial molecular response of epithelial defense may not directly capture the loci underlying phenotypic resistance. Nevertheless, several well-established immune-related genes, such as CXCL8, LTF, TLR2, and BOLA-DRB3, were consistently induced, confirming their relevance in mammary epithelial immunity. These findings prove that milk-derived pbMECs are a powerful non-invasive tool for dissecting functional host responses and complement ongoing efforts toward functional understanding of host–microbe interactions in ruminants. Work contributes functional genomic insights that can be integrated with broader multi-omics frameworks to improve our understanding of mastitis susceptibility and to support future breeding strategies for healthier, more resilient dairy cattle.

Mapping Tryptophan-Derived Metabolites along the Bovine Small Intestinal Tract: An Evolutionarily Distinct Host–Microbiota Adaptation in Ruminants?

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The spatial distribution of metabolites across the gut segments may indicate digestive or microbiota-related specificities, which impact local gut physiology and endocrine functions but also the overall performance and health of ruminants. Gut microbiota extensively metabolizes tryptophan (Trp) into a diverse group of indole metabolites, such as Indole-3-propionic acid (IPA), which act as key signaling molecules regulating epithelial barrier integrity, mucosal immunity, and endocrine regulations. In non-ruminants, indole production is dominant in the distal intestine, whereas in dairy cows, rumen microbial fermentation may severely alter this pattern, which has yet to be elucidated. This study aimed to characterize the spatial distribution of indole metabolites across the small intestine of dairy cows. Intestinal tissue samples were collected from six Holstein ruminant calves (3.5 months old). Metabolome profile of small intestinal segments (duodenum, jejunum, ileum) was measured using MxP® Quant 1000 kit (Biocrates Life Sciences AG, Austria) and liquid chromatography mass spectrometry (LC-MS/MS). Indole-related metabolites, including tryptamine, IPA, Indole-3-acetic acid (IAA), Indole-3-acetamide (IAM), Indole-3-carboxylic acid (I3CA), Indole-3-ethanol (I3E), indoxyl sulfate (Ind-SO4), and Indoleacetaldehyde (IAAld), were selected according to the compound classifications provided by the Biocrates website (https://biocrates.com/). Indole metabolites values below the limit of detection (LOD) were imputed using a 1/5 of the minimum positive values. Differences in indole metabolite abundance across intestinal segments (duodenum, jejunum, ileum) were assessed using a general linear model (GLM) in the MetaboAnalyst V6 platform. Multiple testing was adjusted using the false discovery rate (FDR), and metabolites with FDR < 0.05 were considered statistically significant. IPA (0.06 μM) and Indole-3-acetic acid (0.15 μM) were significantly higher in the duodenum, whereas in the jejunum and ileum their concentrations were below LOD. The concentration of Indoleacetaldehyde (~ 0.003 μM) and indoxyl sulfate (~ 0.11 μM) remained unchanged across intestinal segments. Tryptamine, Indole-3-acetamide (IAM), Indole-3-carboxylic acid (I3CA), and Indole-3-ethanol were detectable across intestinal segments, although at very low concentrations (<LOD), and no significant differences were observed between segments. Metabolomic profiling revealed that indole metabolites are distributed across the entire intestinal tract in dairy cows with certain members showing peak concentrations in the duodenum. The proximal enrichment of indoles represents a distinctive feature compared with non-ruminants where indoles are usually detectable in the distal part and likely reflects a ruminant–specific host–microbiota regulatory mechanisms. Interestingly, IAA and IPA, which were predominant in the duodenum, are well-known for playing a crucial role in strengthening barrier integrity by promoting tight junction expression, also have the ability to enter circulation and impact peripheral tissues. Future studies need to investigate whether indole metabolites function as adaptive signals that fine-tune host regulatory networks such as immune and endocrine responses.

Deciphering the Bovine Nasal Microbiome by reconciling 16S rRNA sequencing and culturomics

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The bovine nasal microbiota is a key line of defense against respiratory pathogens, and numerous studies indicate that its composition can influence the success of pathogen colonization. Yet, the mechanisms underlying the interactions among commensals, pathogens, and the host remain largely unclear. Addressing these knowledge gaps requires not only a refined description of the commensal bovine nasal microbiota but also experimental approaches that enable controlled in vitro investigation of commensals and pathogens' interactions. To tackle these issues, we followed a two-step approach combining large-scale analysis of in vivo nasal microbiota datasets with cultivation of swab-derived microbial communities. First, to generate a comprehensive compositional map of the (healthy) bovine nasal microbiota, we assembled a diverse collection of 654 nasal-swab 16S rRNA sequencing samples. These in vivo datasets span multiple production systems, ages, geographical locations, and diets, allowing us to classify microbial genera based on abundance and prevalence. Despite high overall diversity (> 2,200 detected bacterial genera), the nasal microbiota was dominated by a core set of 10 highly abundant and prevalent genera, including Moraxella, Mycoplasma and Psychrobacter among others. These core genera accounted for ≈ 45% of cumulative abundance across datasets and represent the most stable and consistent members of the bovine nasal microbiota. Beyond this core, we identified two layers of accessory genera: 253 high-prevalence accessory members (HPA), consistently detected and suggesting ecological persistence despite low biomass; and 1,886 low-prevalence accessory members, characterized by sporadic occurrence and low abundance. These findings point towards a layered structure of the bovine nasal microbiota, in which rare taxa may nonetheless contribute to ecological stability, resilience, and host interactions. Second, to assess the feasibility of studying these different taxa in vitro, we cultivated swab-derived communities under multiple growth conditions and used 16S rRNA sequencing to evaluate taxonomic recovery. Under aerobic conditions, core genera exhibited the highest recovery rates (up to 33%), while HPA showed intermediate recovery (up to 17%), and LPA were rarely recovered (typically < 4-6%) across media types. Strictly anaerobic conditions yielded lower recovery rates across all groups. These findings support the hypothesis that dominant taxa are more readily cultivable, while many accessory members likely require specific conditions not reproduced in conventional media. Together, this work provides an updated, high-resolution view of the bovine nasal microbiota and establishes tractable in vitro tools for studying interactions among its members. This integrated framework lays the groundwork for mechanistic studies of how commensals influence pathogen behavior and respiratory health in cattle under in vitro tractable conditions.

Dietary supplementation with buffer and protected amino acids improves milk production and modulates microbiota in heat-stressed primiparous dairy goats

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Background and objective: Heat stress compromises dairy goat productivity and metabolic homeostasis. We hypothesized that dietary supplementation with magnesium oxide buffer and hydroxy analogue of methionine in commercial conditions would improve energy metabolism and modulate microbiota, enhancing milk production under heat stress. Materials and methods: Ninety Murciano-Granadina dairy goats (15 ± 4 DIM) were enrolled in a four-month trial (June-October 2024) at a commercial farm in Granada, Spain. Goats were assigned to either a control diet (CTL, n=45) or a treatment diet supplemented with buffer and protected amino acids (TRT, n=45). Temperature-humidity index (THI) was monitored continuously. Blood samples were collected during heat stress (July, THI=75.6) and thermoneutral (October, THI=66.8) periods. Milk yield and composition were recorded biweekly. Faecal and raw milk samples from primiparous goats (N=19; CTL=11, TRT=8) were analysed for bacterial composition using 16S rRNA gene sequencing. ASVs were identified with DADA2 and taxonomy assigned using Greengenes2 database. Results from blood metabolites were analysed using generalized linear mixed models. Microbiome data underwent quality filtering and rarefaction. Alpha diversity was compared using Wilcoxon tests, beta diversity using PERMANOVA, differential abundance using DESeq2, and discriminant analysis using sPLS-DA. Spearman correlations assessed microbiota-production relationships stratified by treatment. Results: Environmental monitoring confirmed severe heat stress during July-August (THI>80). In primiparous goats, TRT animals produced significantly more milk (+1 kg/day), fat (119.1 vs. 101.1 g/day), and protein (+20 g/day) compared to CTL ($P<0.05$). Blood parameters analyses revealed higher glucose (53.2 vs. 42.9 mg/dL, $P<0.001$) and lower non-esterified fatty acids (NEFA; 0.46 vs. 0.61 mmol/L, $P<0.05$) in TRT goats, indicating improved energy balance. Milk microbiome analysis showed trends toward reduced alpha diversity in TRT (Pielou evenness: $P=0.051$; phylogenetic diversity: $P=0.062$), while beta diversity did not differ significantly between groups. DESeq2 identified seven genera less abundant in TRT milk (FDR<0.05), predominantly Actinobacteria (Pseudonocardiaceae family: Prauserella, Saccharopolyspora; and Nocardiopsis), suggesting reduced environmental contamination or altered mammary ecology. Notably, treatment moderated microbiota-production relationships: Staphylococcus-protein yield correlation was positive in CTL ($\rho=0.66$) but negative in TRT ($\rho=-0.49$), while Stenotrophomonas-fat yield shifted from negative in CTL ($\rho=-0.47$) to positive in TRT ($\rho=0.89$), indicating treatment-dependent host-microbiome interactions. Faecal microbiota showed significant community separation by sPLS-DA, with 12 differentially abundant taxa including decreased Akkermansia, Alistipes, and Cryptobacteroides; and increased Ruminococcus_E and Bifidobacterium in TRT goats. Conclusion: Dietary supplementation with buffer and protected amino acids improved milk production and energy metabolism specifically in primiparous dairy goats under heat stress. These improvements were associated with distinct shifts in both mammary and faecal bacterial composition, with milk microbiota showing reduced environmental taxa and treatment-dependent correlations with production traits. These findings support the hypothesis that nutritional supplementation improves productive performance through enhanced energy balance and microbiota modulation, with effects most pronounced in primiparous goats.

Exploring Bacterial Defluorination of Sodium Fluoroacetate in Rumen Fluid

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Livestock mortality associated with sodium fluoroacetate (SF)-producing plants, particularly Dichapetalum cymosum, remains a major constraint to sustainable livestock production in southern Africa. Conventional mitigation strategies, such as veld management and herbicide application, are often economically and logistically impractical across extensive rangelands. Although symptom-reversal therapies and genetically modified SF-detoxifying bacteria have been developed, their effectiveness is constrained by treatment timing and regulatory limitations. Recent evidence indicating the presence of naturally occurring SF-degrading rumen bacteria offers a promising, biologically based alternative. This study evaluated the feasibility of developing a natural ruminal detoxification strategy combining SF-degrading bacterial isolates with tricarboxylic acid (TCA) cycle intermediates to enhance detoxification efficiency. Using an in vitro Daisy II incubation system, teff hay was subjected to four treatments: (T1) control; (T2) basal diet + Pigmentiphaga kullae and Ancylobacter dichloromethanicus (probiotic treatment); (T3) basal diet + sodium citrate and arginine (TCA intermediates); and (T4) basal diet + probiotics + TCA intermediates. Rumen fluid from cattle served as inoculum. Samples were collected at 0, 12, 24, and 48 h to determine dry matter degradability (DMD) and rumen pH. Bacterial community composition was characterised by targeted 16S rRNA metagenomics. Rumen pH remained within the physiological range (6.0–7.0) across all treatments, indicating no adverse effects on ruminal fermentation conditions. However, DMD differed significantly ($P < 0.05$) among treatments. TCA supplementation alone slightly reduced degradability relative to the control, whereas the combined probiotic and TCA treatment (T4) mitigated this decline. Microbial profiling revealed treatment-specific shifts in community structure, with Bacillus, Pseudomonas, Ruminobacter, and Lysinibacillus among the dominant genera. Notably, the combined treatment enhanced microbial diversity and promoted greater community stability compared to TCA supplementation alone. Collectively, these findings support the potential of integrating naturally occurring SF-degrading rumen bacteria with targeted nutritional supplementation as a complementary strategy to mitigate SF toxicity. Further optimisation of inoculum dosage, substrate composition, and in vivo validation is warranted to advance practical application under field conditions.

Influence of post-birth management and milk replacer feeding level on early life performance, health and behaviour of dairy calves

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The early life of a calf involves a complex mix of environmental and management factors that can influence long-term production efficiency, growth, resistance to disease, and phenotypic resilience to changing environmental conditions. This study aimed to determine the impact of divergent early life management practices on calf behaviour during the first week of life and performance during the first 10 weeks of life. Biological samples collected at key times will be further used to characterise the establishment of microbiomes as well as determine their influence on key performance indices. Forty-eight Holstein heifer calves were allocated in a 2 × 2 factorial design (n = 12 calves per treatment), with 2 birthing protocols (Remain vs Remove) and 2 × milk replacer (MR) feeding levels (Low vs High). Calves were balanced on each of the four treatments, as far as possible, for birthweight, sire, dam production parameters and dam genetics. Remain calves stayed with their dam in the calving pen for 24 h (22-26 h) following birth while Remove calves were taken from the dam immediately and placed in an individual pen. Low MR calves received 4 L/day MR, with High MR calves offered a maximum of 8L/day until weaning at 56 days of age. Calves were individually penned from 24h post birth until the end of the study and provided with ad lib access to water and starter concentrate feed from 5 days of age. Liveweight was recorded at 24 h of age and weekly according to individual calf age between 7-70 days of age. Activity monitors were affixed to calves throughout the experimental period. Data was analysed using GenStat (version 21.1, VSN International Ltd). Feed intake, liveweight and activity data were analysed using a repeated measures model with birthweight, time point, birthing protocol and MR feeding level along with associated interactions fitted as fixed effects where applicable. Average daily gain (ADG) was subject to REML variance components analysis using a linear mixed model with fixed effects of birthing protocol, MR feeding level and associated interactions. Probability of $P<0.05$ was selected as the level of significance, and data was then subject to Fisher's protected least significant difference test where applicable. Daily MR intake was increased in Remove compared to Remain calves ($P<0.001$), however, at a practical level this only represented an average increase of 52 ml/day. Calves offered 4 L/day of MR consumed an average of 111 g DM/day more starter than High MR calves during the pre-wean period ($P=0.002$), however this effect was not evident in the post-wean period ($P=0.770$). Birthing treatment had no effect on starter intake during the pre-or post-wean periods, or on liveweight ($P=0.404$), or ADG over the first 10 weeks of life ($P=0.142$). Both lying time and motion index in calves were significantly impacted by birthing treatment in the first 24 hours following birth, whereby motion index was increased and average lying time for each 15-minute period was reduced in Remain calves compared to Remove calves ($P<0.001$). However, no further differences in lying behaviour parameters were observed during the pre-wean period ($P>0.1$). Removal at birth compared with allowing the calf to remain with the dam for 24 h had limited impact on calf behaviour after the initial 24 h period. Future work should examine the impact of extended periods of cow-calf contact in the days following birth. Results highlight that calves can be offered higher volumes of milk replacer in the pre-wean period without affecting post-wean starter intake and with a positive impact on growth.

Is the Pig Faecal Microbiota Shaped by Host Genetics?

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A key aspect for understanding the variability in gut microbial composition is estimating the host genetic component associated with it. Therefore, this study aimed to estimate the heritability of faecal bacterial genera abundances at the genus level, and of α-diversity indices derived from amplicon sequence variant (ASV) profiles in pigs. Based on these findings, regional heritability mapping (RHM) was applied to identify genomic regions that are associated with pigs' bacterial genera. The study design included 450 Pietrain × Landrace F1 crossbred pigs with a defined family structure. The animals were kept under standardised individual housing and feeding conditions from the 11th week of life onwards. All animals were genotyped for 48,525 SNPs and faecal microbiota composition was characterised by 16S rRNA gene amplicon sequencing. Faecal microbiota were profiled at 16 weeks of age. Relative abundances were initially summarised across 325 bacterial genera; following prevalence filtering (minimum 20% of animals), 150 genera were retained for analysis. ASV-level data were subjected to quality and minimum occurrence filtering, yielding 6,344 ASVs for subsequent analyses. Genomic mixed linear models were used to estimate variance components for heritability estimation for individual bacterial genera and α-diversity indices. To assess α-diversity, richness was calculated as the count of genera with non-zero relative abundance, while the Shannon index was calculated to assess overall community diversity. Seventy-six of 150 genera revealed significant heritability estimates ($p < 0.05$) ranging from 0.11 (SE = 0.07) to 0.45 (SE = 0.10). For 14 genera, a heritability greater than 0.30 was estimated. Results for the α-diversity showed a heritability of 0.11 (SE = 0.07) for richness and 0.11 (SE = 0.08) for the Shannon index. These findings indicate that a subset of faecal bacterial genera is substantially influenced by host genetics. Genera that showed a stronger genetic component were further selected and RHM was conducted to identify genomic regions contributing to host genetic control of these genera. The separation of genetic and non-genetic factors shaping the pig faecal microbiota is crucial when the microbiota is considered as biomarker or target in breeding programmes. This study demonstrates a pipeline for assessing heritability in the microbiome and reports initial estimates to be substantiated in larger cohorts.

Comprehensive analysis of rumen-derived bacteriocin-producing Lactococcus lactis for Streptococcus biofilm control strategies

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The rumen is a highly complex microbial ecosystem composed of interlinked ecological niches that collectively drive ruminant nutrition and health. Within this system, lactic acid bacteria (LAB) act as crucial facultative anaerobes that ferment carbohydrates into lactic acid, serving as an intermediate substrate for propionate-producing bacteria—providing a primary energy source for ruminants. Beyond their metabolic roles, several LABs, including Lactobacillus, Streptococcus, and Enterococcus, produce bacteriocins that influence microbial community structure and suppress opportunistic pathogens. However, the diversity and functional relevance of rumen-derived bacteriocin-producing LABs remain insufficiently characterized. In this study, ten strains of Lactococcus lactis subsp. lactis were isolated from rumen fluid of Hanwoo cows (Anseong, South Korea), fed a 50:50 (DM) rice straw-concentrate diet. Bacteriocin characterization included stability assessments across a range of temperatures (60°C – 121°C), pH levels (2 – 10), and proteolytic enzyme treatments (proteinase K, pepsin, and trypsin), determination of minimum inhibitory activity via checkerboard assay, and subsequent evaluation of biofilm inhibition, with statistical significance analyzed using one-way and two-way ANOVA with post hoc multiple comparisons tests ($p < 0.05$). All isolates exhibited inhibitory activity against Streptococcus equinus KCTC 21172 (MIC $\geq$ 400 AU/mL) and Listeria monocytogenes strains (MIC $\geq$ 100 AU/mL). Lactococcal bacteriocins significantly inhibited S. equinus KCTC 21172 biofilm formation ($p < 0.05$) and effectively disrupted pre-formed streptococcal biofilms (46.54 ± 7.37% to 83.50 ± 1.40%), highlighting their potential applications in biofilm prevention of specific rumen-microbial consortia, associated with subacute ruminal acidosis (SARA) and opportunistic pathogenesis. Biochemical characterization revealed properties consistent with nisin-like bacteriocins. Complementary meta-analysis of publicly available lactococcal genomes ($n = 99$) revealed substantial bacteriocin diversity for L. lactis subsp. cremoris (i.e., lactococcins, garvicin, and enterolysin) and subsp. lactis (i.e., lactococcins, nisin, and enterocin), likely reflecting ecological adaptation. Statistical analysis of bacteriocin gene distribution using Fisher's exact test (two-tailed) with Benjamini–Hochberg correction for multiple comparisons revealed that garvicin Q and lactococcin A were significantly enriched in L. lactis subsp. cremoris, whereas nisin and sactipeptides were significantly associated with subsp. lactis ($q < 0.05$), supporting subspecies-specific bacteriocin profiles. Collectively, these findings demonstrate that rumen-derived lactococci are potent producers of antimicrobial peptides and represent promising candidates for modulating rumen microbiota, controlling opportunistic pathogens, and mitigating biofilm-associated rumen disorders.

Effects of selected rapeseed meal cultivars in low-protein diets on performance and oxidative stress in dairy cows and their calves during the periparturient period

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Dairy cows and calves experience acute oxidative stress and inflammation around the calving. A previous study showed that two rapeseed (R) cultivars 00 (M: Mambo; B: Bonanza) improved the redox status of Ob-Ob mice through specific polyphenols and glucosinolates. This study aimed to evaluate the effects of rapeseed meals of these two cultivars, on the performance, immunity, and oxidative stress of dairy cows and their calves from week (wk) 4 before to wk 5 after calving. Multiparous Holstein cows (n = 45) were assigned to 4 groups of 10-12 cows. Each group was fed a maize silage-based diet containing fixed amounts of dry matter (DM, kg pre/post-partum) of toasted lupin (L: 0.88/1.2), overheated (140°C) M meal to alter specific metabolites (HM: 1.5/2.3), or low-temperature (110°C) M or B meals (1.5/2.1). The Protein Digestible in the Intestine (PDI) of the HM diet was higher than in L, M, B diets (81.6 vs. 70.9 / 96.6 vs 87.8, respectively). Net energy was similar across the diets (1.44 Mcal/kg DM). Calves (12/diet) received maternal colostrum (2.9 ± 0.8 kg) and milk for 2 days (d), then fixed amounts of pooled milk from cows in matched dietary groups. until wk 5. Blood was collected 2 wk before calving (cows), and after calving, on d1 (cows) or d2 (calves, wk 1), and wk 3 and 5 (cows and calves). Data were analysed by ANOVA with diet, wk and diet × wk as main effects and animal as a random effect, wk as a repeated measure and covariates for milk parameters. Orthogonal contrasts were performed: L vs. R, M&B vs. HM, and M vs. B. DM intake (DMI) of prepartum cows was 12.6 ± 0.4 kg/d (fixed offers). During lactation, DMI was lower for L vs. R diets (21.7 vs. 23.9 kg; $P < 0.05$), which led to lower yields of milk (36.3 vs. 39.1 kg/d), protein (1096 vs. 1241 g/d), fat (1461 vs. 1623 g/d) and plasma concentrations of most essential amino acids (AA). Despite the lower PDI supply in M&B vs. HM diets, cows fed M&B or HM produced similar milk and protein yields resulting in a greater PDI efficiency (0.79 vs. 0.72; $P < 0.05$) in the M&B diets. This suggests reduced AA catabolism and urinary N losses. Milk somatic cell scores were the lowest for M cows (M vs. B: 1.3 vs. 2.5, $P= 0.07$) and lactose concentration was the highest in M&B cows (47.5 g/kg vs 46.5 g/kg; M&B vs. HM: $P = 0.08$), suggesting reduced mammary inflammation associated with feeding M and B diets. Calves' total milk intake (247 ± 9 kg) and BW gain (0.76 ± 0.13 kg/d) were not influenced by treatments. Similar amounts of α and γ-tocopherols (600 and 757 ± 50 mg/d) were ingested for L vs. R diets. Cows fed L vs. R tended ($P < 0.1$) to have lower plasma hydrogen peroxides (dROM) and reduced glutathione reductase activity (antioxidant enzyme) at wks 3 and 5 ($P < 0.05$). Calves receiving L vs. R milk also had lower plasma dROM ($P = 0.02$) and greater total antioxidant capacity (FRAP, $P = 0.06$). This suggested lower oxidative stress in both L cows and calves. Cows fed M&B vs. HM diets had lower ($P < 0.06$) plasma dROM, and plasma glutathione was higher in their calves ($P = 0.01$). Calves fed with M vs. B milk had higher plasma haptoglobin, and lower activity of superoxide dismutase ($P < 0.05$). Plasma concentrations of immunoglobulins G and M in calves were unaffected by maternal diets. In conclusion, in low PDI diets, functional metabolites in toasted lupin or rapeseed cultivars have only subtle effects on oxidative stress in cows and calves but low heated meals from rapeseed cultivars maintained DMI and milk production.

Supplementing a Bacillus-based probiotic to mitigate acidosis in beef cattle consuming a high-concentrate diet

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Bacillus-based direct-fed microbials are proposed to modulate ruminal fermentation and microbial ecology, but their effects on ruminal responses to an acidosis-inducing diet are not fully understood. This experiment compared ruminal fermentation, nutrient degradability, and performance responses of beef heifers receiving an acidosis-inducing diet and supplemented with a Bacillus-based probiotic (BAC; Bacillus licheniformis + B. subtilis; Bovacillus™; Novonesis, Lyngby, Denmark). Fifteen ruminally-cannulated heifers were ranked by pre-trial body weight (BW) and assigned to 3 groups of 5 heifers each. Groups were enrolled in a replicated 3 × 3 Latin square design containing 3 periods of 21 days, and a 14-day washout interval between periods. During each period, heifers had ad libitum access to water and were offered a forage-based diet from day 0 to 6 and an acidosis-inducing diet from day 7 to 21 at 2% of BW (DM basis), in equal proportions at 0700 and 1600 h. Groups were assigned to receive no BAC supplementation (CON; n = 15), BAC supplementation at 3 g/heifer daily from day 7 to 21 (BAC; n = 15), or BAC supplementation at 3 g/heifer daily from day 0 to 21 (BACPRE; n = 15). The BAC dose was mixed with dried distillers' grain and manually blended into the diet at each feeding. Heifer unshrunk BW was recorded on days -3, -2, and -1 relative to the beginning of each period and averaged for initial BW. Final BW was calculated by averaging heifer BW on days 19, 20, and 21. Rumen fluid samples were collected on days 0, 7, 14, and 21 of each period, immediately prior to (0 h) and at 3, 6, and 9 h relative to the morning feeding. Results obtained on day 0 were averaged and used as independent covariate in each respective analysis. Dietary dry matter and nutrient degradability in the rumen were evaluated from day 17 to 20 using Ankom bags. No treatment differences were detected ($P \geq 0.20$) for heifer BW gain, feed intake, or gain:feed ratio. No treatment effects or interactions containing the treatment effect were detected ($P \geq 0.33$) for rumen pH. No treatment differences were detected ($P \geq 0.15$) for concentrations of individual or total volatile fatty acids on day 7. On day 14, heifers assigned to BAC had less ($P = 0.03$) butyrate concentration compared with BACPRE and CON heifers, whereas no other differences were detected ($P \geq 0.41$) for individual or total volatile fatty acids. On day 21, heifers assigned to BAC and BACPRE had greater ($P = 0.03$) valerate concentration compared with CON heifers, whereas no other differences were detected ($P \geq 0.36$) for individual or total volatile fatty acids. Heifers assigned to BAC and BACPRE had greater ($P \leq 0.02$) diet dry matter disappearance rate (4.15 and 4.02 vs. 2.99 %/h, respectively; SEM = 0.33) and effective degradability (61.1 and 60.0 vs. 57.6%, respectively; SEM = 0.10) compared with CON heifers. In summary, supplementing BAC to beef heifers receiving an acidosis-inducing diet increased ruminal dry matter disappearance rate and effective degradability, but did not modify rumen pH or overall volatile fatty acid concentrations.

Effects of inoculation of corn silage with Lactobacillus buchneri and Lactococcus lactis on silage quality and stability, and production performance and efficiency in beef cattle

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This study evaluated the effect of a novel silage inoculant based on Lactobacillus buchneri and Lactococcus lactis on corn silage quality, conservation, and aerobic stability, as well as its impact on beef cattle performance, production efficiency, and health. At harvest, corn silage from the same fields was evenly divided into two bunker silos and either treated or not with the inoculant (SiloSolve® FC, Chr. Hansen A/S, Hørsholm, Denmark) at 2g/ton of fresh silage. After a 60-days ensiling period, the two silages were fed to 292 Charolais beef bulls, blocked by arrival weight and divided evenly in two groups: i) Control (146 bulls), receiving basal diets containing control untreated silage; ii) Treatment (146 bulls), receiving basal diets containing the inoculated silage. The trial covered the entire fattening period of 186 days. Silage samples were collected monthly and analyzed for chemical composition, fermentative profile, mycotoxins content, and biogenic amines (putrescin, cadaverine and histamine). Individual weights were taken at the beginning and end of the trial to calculate the average daily gain (ADG). The feed intake was measured monthly, and the feed conversion ratio (FCR) was calculated. Health was monitored daily, with particular attention to digestive disorders and nutrition-related lameness. Apparent total tract digestibility (aTTD) was evaluated monthly per pen using a portable near-infrared system by comparing diet and fecal composition. Slaughtering performances were also recorded. The data were analyzed using SAS software with specific models applied to each parameter. No differences were observed in the chemical composition of the two silages. Conversely, the fermentative profile was improved in treated silage, showing lower pH (3.65 vs 3.93), butyric acid (0.01 vs 0.23 % on dry matter), ammonia (NH3) (929.71 vs 1640.43 ppm), and higher lactic and acetic acids (7.23 and 3.73 vs 5.95 and 2.46 % on dry matter) ($P < 0.0001$). Mycotoxin concentrations and biogenic amine levels were also significantly reduced in treated silage ($P < 0.0001$). Those trends were also stable over time. Treatment improved ADG (1.528 vs 1.475 kg/head/day) ($P < 0.0001$) and showed a tendency toward higher final body weight (722.25 vs 716.17 kg) ($P = 0.064$) and carcass weight (429.9 vs 425.94 kg) ($P = 0.055$). Feed intake was not affected, whereas FCR was significantly improved in the Treatment group (7.58 vs 8.10) ($P < 0.0001$). Carcass fatness score was also improved, with a higher proportion of carcasses classified as fatness score 3 in the Treatment group (33.33 vs 19.05%) ($P = 0.004$). The average aTTD of NDF (52.18 vs 50.79%) ($P=0.0001$), cellulose (54.06 vs 50.01%) ($P = 0.045$) and starch (96.75 vs 95.68%) ($P<0.0001$) were significantly improved by the Treatment, with also stable results over time. A tendency toward a reduction in nutrition-related non-traumatic lameness was also observed (1.59 vs 5.56%) /$P = 0.068$). The inclusion of the inoculant improved significantly the stability and safety of corn silage, leading to enhanced digestibility at the rumen and potentially contributing to a more stable ruminal environment, ultimately supporting improved production performance in beef cattle.

Effects of Biochar Supplementation on Milk Yield, Milk Composition, Milk Fatty Acids and Health in Dairy Cows

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In dairy cows, biochar supplementation has been linked to improved health, better feed conversion, and higher milk yield. However, previous studies often relied on small sample sizes or limited observation periods providing only a snapshot of short-term effects. In this study, we investigated the long-term effects of variable biochar supplementation on milk yield, milk composition, milk fatty acid concentrations and health parameters of dairy cows. A feeding trial was conducted from May to November in 2024 on a conventional dairy farm located in eastern Germany. The biochar feeding was divided into three consecutive phases. One group of dairy cows (n = 132–194 per phase) received biochar in increasing concentrations (0.5%, 1%, 2% of feed), whereas the control group (n = 144–185 per phase) received the same feed without added biochar. Daily milk yield of each cow was recorded by a milking robot. In each phase, the milk composition (fat, protein, lactose, urea) was analysed in a routine milk inspection. On day 43 of the second phase and on day 4 of the third phase during the feeding period, milk samples were collected to determine milk fatty acid concentrations using gas chromatography. Health data, including the number of affected cows, disease incidence, and administered medications (analgesics and antibiotics), were recorded throughout the study period. The data was analysed using R version 4.5.1 (R Core Team, 2025). A linear mixed-effects model was fitted to account for repeated measurements within animals and including treatment, lactation group, lactation stage, and individual baseline values as fixed effects. Considering milk yield, significant effects were observed only in the first phase. Cows in their first lactation produced an estimated 1.06 kg/d more milk in late lactation (days in milk ≥ 105; $P = 0.04$), and cows in their fourth or higher lactation produced an estimated 2.78 kg/d more milk in early lactation (days in milk < 105; $P = 0.03$) compared with the control animals. Regarding milk composition, protein content was affected only in the last phase, when 2% biochar was added to the feed. In this phase, the biochar fed group showed an estimated 0.10% higher milk protein content than the control group ($P < 0.01$). For evaluation, the analysed milk fatty acids were grouped by degree of saturation and carbon chain length. Differences were detected only on the first sampling day, when the biochar group showed higher estimated concentrations of monounsaturated fatty acids (1074 ± 49 vs. 878 ± 55 mg/100g milk, $P < 0.01$) and polyunsaturated fatty acid (134 ± 6 vs. 121 ± 6 mg/100g milk, $P = 0.04$) than the control group. Additionally, linoleic acid ($P = 0.01$) and alpha-linolenic acid ($P = 0.04$) were higher in the biochar group, again only on the first sampling day. Apart from more frequent analgesic treatment in the biochar group during the first phase ($P = 0.03$), and a higher estimated somatic cell count of the biochar group in the second phase ($P < 0.01$), no effects of biochar on health parameters were observed. In summary, the results suggest that biochar supplementation had no consistent significant effect on milk yield, milk composition or health indices. Minor changes in milk fatty acid concentrations were observed, but no clear overall pattern emerged. Although a temporary increase in milk protein content in the third phase indicates a possible effect on rumen microbiota and amino acid supply for milk production, this was not yet investigated further. At the same time, biochar does not appear to compromise milk production either.

Effects of supplementing 25 OH vitamin D3 on immune markers and colostrum quality in Holstein cows during the transition period, and on plasma vitamins and metabolites in their newborn calves

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This study investigated impact of dietary supplementation of 25-OH-D3 (HyD) on (I); plasma acute-phase proteins, cytokines, and immunoglobulin (Ig) concentrations in colostrum of multiparous Holstein cows from three weeks before calving through 21 days in milk, and (II); plasma levels of fat-soluble vitamins, Ig, minerals, and metabolites in their newborn calves. A total of forty-two cows were allocated to one of two treatments: vitamin D3 (D3) or HyD supplementation. From −21 d relative to expected calving until parturition, cows in the D3 group received 0.475 mg/d (19,000 IU/d; 0.035 mg/kg DM) of vitamin D3, whereas HyD cows received 1.45 mg/d (58,000 IU/d; 0.108 mg/kg DM) of HyD. From calving to 21 DIM, D3 cows continued to receive 0.625 mg/d of vitamin D3 (25,000 IU; 0.024 mg/kg DM). During the same period, HyD cows received the same D3 dose plus 1.0 mg/d (40,000 IU of HyD; 0.056 mg/kg DM). Blood samples were collected from the tail vein on days −7 (6 ± 2 d), −3 (2 ± 1 d), 0 (13 ± 8 h after calving), 1 (36 ± 11 h after calving), 10 (9 ± 2 d), and 20 (16 ± 2 d) relative to parturition. These samples were analyzed for plasma concentrations of acute-phase proteins (haptoglobin [HAP] and serum amyloid A [SAA]), and cytokines including tumor necrosis factor-α (TNF-α), interferon-γ (IFN-γ), and interleukin-10 (IL-10). After calving, cows were milked in the calving pen, and a colostrum sample was collected (2.2 ± 1.9 h after calving; mean ± SD). Calves were then immediately fed colostrum from their own dam. A blood sample was also collected from each calf via jugular vein at 48 ± 3 hours of age (mean ± SD). For maternal plasma TNF-α concentrations, a treatment × time interaction was detected ($P < 0.01$), and cows receiving HyD showed reduced TNF-α levels before calving compared with those given D3, but this difference diminished around parturition and the two groups reached comparable concentrations by 20 days in milk. No treatment effect was observed for plasma Hap (0.54 g/L), SAA (17.8 pg/mL), IFN-γ (333 pg/mL), or IL-10 (177 pg/mL; means across treatments). Newborn calves from cows supplemented with HyD showed greater plasma 25-OH-D3 concentrations compared with calves from the D3 group (13.9 vs. 8.9 ng/mL; $P < 0.01$). In contrast, plasma levels of 25-OH-D2 (1.7 ng/mL), vitamin A (0.16 µg/mL), and vitamin E (2.2 µg/mL; means across treatments) in the calves did not differ between treatments. A positive linear association was observed between the calves' plasma 25-OH-D3 concentrations and those measured in their dams ($Y = 6.8 + 0.06 X$). Colostrum volume (4.1 L), Brix value (20.7%), and colostrum concentrations of IgA (1,113 µg/mL), IgM (5,614 µg/mL), and IgG (101 mg/mL; means across treatments) did not differ between cows receiving D3 and those supplemented with HyD. Consistent with these findings, the calves' plasma levels of IgA (326 µg/mL), IgM (25 µg/mL), and IgG (23 mg/L; means across treatments) also showed no treatment differences between the two groups. In conclusion, HyD supplementation had minimal effects on acute-phase proteins and cytokine responses in transition cows. Supplementing HyD during close-up period led to enhanced 25-OH-D3 levels in calves at birth; however, no associated influence on immunoglobulin concentrations in colostrum or calf plasma was noted. Significance of elevated 25-OH-D3 in calf plasma in relation to active immune development remains to be investigated.

Effects of a rumen-protected choline prototype on milk yield and composition, liver triglyceride, and blood parameters for early lactation Holstein cows exposed to feed restriction

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During early lactation, dry matter intake (DMI) lags behind milk production, causing cows to enter a negative energy balance. This deficit necessitates mobilization of adipose reserves as non-esterified fatty acids (NEFA) to promote milk production. Although NEFA can be fully or partially oxidized for energy, excessive mobilization rates can exceed metabolic capacity of the liver and export of NEFA as very low-density lipoproteins (VLDL), resulting in triglyceride (TG) accumulation in the liver. Choline is a precursor for phosphatidylcholine, a key component of VLDL. Greater VLDL production enhances hepatic clearance rates of NEFA, limiting the risk for TG deposition. As dietary choline supply is often inadequate during early lactation, dietary supplementation with rumen-protected choline (RPC) may be of use for dairy cows. The objective of this study was to determine the effect of a RPC prototype on DMI, milk yield and composition, liver TG concentration, and blood metabolites in 16 early-lactation Holstein cows (DIM = 44 ± 5 d, initial milk yield = 45.6 ± 8.8 kg/d) undergoing a feed restriction challenge. The study used a randomized complete block design consisting of 14 d of adaptation to RPC, 5 d of baseline (BASE), 5 d of feed restriction (FR; 40% of ad libitum intake), and 10 d of recovery (REC). Treatments included a negative control (CON; n = 8) or a daily RPC dose (RPC; 27 g/d, providing 12 g/d of choline ion; n = 8) mixed with 1 kg of the TMR 30 min prior to feeding. All cows were fed the same barley silage-based TMR. Blood samples were collected on d 1, 19, 21, 23, 25, 26, 27, 29, 31, and 34 relative to the start of the study. Liver biopsies were performed on d 17, 25, and 34. Cows were milked 3× daily and milk yield from d 15 to 34 were used for statistical analysis. Composite milk samples from four time-points (d 17-19, 22-24, 25-27, and 32-34) were analyzed for composition. Data were analyzed using the GLIMMIX procedure in SAS v9.4, with treatment, time, and treatment × time as fixed effects, and block and cow as random effects. Milk, DMI, and liver data were analyzed by phase (BASE, FR, REC) with REC further split into two components (REC1 and REC2) for milk and DMI. Blood data were analyzed by day, with d 1 of study (before treatment offered) as a covariate. There were no treatment × time interactions detected for any variables ($P \geq 0.14$). As imposed, DMI was greatest during BASE (29.0 kg/d), was 39% of BASE during FR (11.0 kg/d), and gradually increased during REC (24.5 kg/d; phase, $P < 0.01$). There was no effect of treatment on DMI ($P = 0.52$), but RPC numerically increased DMI during REC. While milk yield did not differ by treatment ($P = 0.35$), cumulative yield was numerically greater for RPC than CON. Milk yield was greatest during BASE (49.4 kg/d), least during FR (34.0 kg/d), and increased during REC (38.8 kg/d; phase, $P < 0.001$). Milk fat percentage increased during FR (5.1%) compared with BASE (3.6%) and REC (4.1%; phase, $P < 0.01$), with no effect of RPC ($P = 0.40$). Blood concentrations of NEFA were lowest in BASE (355 µEq/L), increased during FR (1,710 µEq/L), and decreased during REC (769 µEq/L; day, $P < 0.01$). Liver TG concentrations were lowest during BASE (14.1 µM), highest during FR (486.6 µM), and decreased during REC (41.3 µM; phase, $P < 0.001$). There was no treatment effect on blood NEFA ($P = 0.96$) or liver TG ($P = 0.93$). Supplementation with the RPC prototype to cows exposed to a FR challenge did not affect DMI, milk yield and composition, liver TG, or NEFA. It is possible that the RPC evaluated could not overcome the metabolic stress imposed with severe FR.

Breed impacts voluntary intake of hydroxychloride and sulphate sources of trace minerals in beef heifers

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This study investigated the influence of breed (Bos taurus vs. Bos indicus) on the preferential choice and voluntary selection of two different sources of Cu, Mn, and Zn when incorporated into a white salt-based trace mineral supplement (day 0 to 55; Exp. 1) and a protein-based trace mineral supplement (day 56 to 112; Exp. 2). On day 0, Nellore and ½ Angus × ½ Nellore heifers (n = 20/breed) were categorized by breed, body weight (347 ± 82 kg), and age (12–30 months) and assigned to individual drylot pens. Each experiment included a 28-day washout phase followed by a 28-d evaluation of voluntary intake phase. During the preferential intake phases, heifers were given simultaneous access to sulphate (SUL) and hydroxychloride (HYD) sources of Cu, Mn, and Zn, which were blended into salt-based trace mineral supplements from day 28 to 55 (Exp. 1) and protein-based trace mineral supplements from day 84 to 112 (Exp. 2). From day 0 to 112, heifers had unrestricted access to Tifton 85 (Cynodon sp.) hay. A breed × source × day interaction was observed ($P \leq 0.05$) for daily trace mineral intake from day 28 to 56 and day 84 to 112. Angus × Nellore heifers consumed more SUL than HYD supplements between days 31 and 33 ($P = 0.02$) but preferred HYD over SUL from days 37 to 42 ($P \leq 0.02$). Conversely, Nellore heifers exhibited a higher intake of HYD compared to SUL between days 31 to 33 and 43 to 51 ($P \leq 0.05$). Across both breeds, intake of protein supplements containing HYD was greater ($P \leq 0.05$) than those with SUL from day 84 to 112, with the difference being more pronounced ($P \leq 0.04$) in Nellore than in Angus × Nellore heifers. Overall, Nellore heifers strongly preferred mineral and protein supplements containing hydroxychloride compared to sulphate sources. Regardless of breed, hydroxychloride supplementation promoted greater voluntary intake and minimized variability in consumption compared to sulphate-based minerals.

Post-weaning optimization by using fibrous concentrate and essential oils supplementation in lambs

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Improving rumen fermentation and health during the post-weaning (PW) period while maintaining animal performance and reducing feeding costs represents major challenges in intensive ruminant productions. This study hypothesized that the use of a forage-free fibrous concentrate diet and supplementation with a commercial essential oil (EO) blend (Agolin® Ruminant; 200 mg/kg DM) could optimize the PW process in lambs. A total of 56 weaned male lambs (40 days-old) were randomly distributed into 28 pens and allocated to four experimental treatments according to a 2 × 2 factorial design: a conventional concentrate plus barley straw without (CTL−) or with EO supplementation (CTL+), and a fibrous concentrate without straw supplementation without (FIB−) or with EO supplementation (FIB+). Rumen fermentation and microbiota were evaluated at 2 weeks PW; blood health indicators were measured at 1, 3, and 10 weeks PW; feeding behavior was recorded during week 5; and animals were slaughtered at 11 weeks PW in a commercial abattoir. Results indicated no differences in DMI during the PW period. The FIB diets resulted in a more homogeneous DMI throughout the day, whereas CTL lambs showed higher DMI during the morning. The FIB diet increased total VFA concentration (+33%) and reduced rumen pH, promoting a shift toward propionate production (+6%) during PW. Microbiologically, the FIB diet was associated with the absence of rumen protozoa, reduced methanogen abundance (−4%), and increased abundance of propionate producers such as the family Selenomonadaceae (+98%), while decreasing several fiber-degrading taxa within the phyla Bacillota_A and Bacillota_I. Lambs fed the FIB diet exhibited higher blood β-hydroxybutyrate concentrations (+32%), a lower glucose-to-β-hydroxybutyrate ratio, and reduced urea-N concentration (−29%), indicating increased rumen fermentative activity and improved nitrogen utilization. Anatomically, lambs fed the FIB diet had a lighter rumen epithelium color ($P = 0.036$), suggesting a lower degree of keratinization than CTL lambs at 11 weeks PW. The EO supplementation promoted favorable changes in rumen fermentation during PW, including a tendency to increase propionate concentration (+11%) and the D/L lactate ratio, while decreasing NH_3-N concentration (−52%). EO supplementation exerted antimicrobial effects characterized by a reduction in rumen bacterial richness (−22 ASVs), along with decreases in low-abundance taxa, including the phylum Spirochaetota. Moreover, EO supplementation decreased methanogen richness and the relative abundance of methanogen reads, but only in lambs fed the FIB diet. EO supplementation had no effects on blood health indicators or feeding behavior. Average daily gain, feed efficiency, carcass weight, and diarrhea index were not affected by the experimental treatments. During the entire experiment, concentrate intake was similar across treatments; however, CTL lambs additionally consumed straw (7% of total DMI), resulting in greater total DMI and higher feeding costs (+2.05 EUR/lamb). Overall, this study demonstrates that the post-weaning period can be optimized through targeted nutritional interventions. EO supplementation shifted rumen fermentation toward a potentially more efficient profile, whereas the forage-free FIB diet boosted the rumen fermentation and reduced feeding costs without compromising health or productivity. The combination of these strategies may therefore represent a synergistic approach to enhancing rumen function and profitability in intensive lamb production.

Effects of oilseed supplementation on organoleptic and nutritional quality of Rubia Gallega veal

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Dietary supplementation with essential oils derived from hydrolysed R. communis oilseed (Bovino Plus ®) was evaluated as a nutritional strategy to modulate Longissimus th. muscle on physicochemical and fatty acid analyses. Twenty-four Rubia Gallega male calves from Mabegondo Agricultural Research Centre herd were randomly assigned to one of three treatment groups for 90 days: control (G0), 10 mL/animal/day of essential oils supplement (G1) or 20 mL/animal/day of essential oils supplement (G2). Animals were fed indoors with concentrate and hay ad libitum. The intake of concentrate was calculated daily for each group as the difference between the amount offered and the amount refused (mean daily intake 6.08 kg DM /calve/day). Animals were slaughtered at 312 days of age, with mean slaughter live weights of 449.25 kg (G0), 437.25 kg (G1), and 465.87 kg (G2), and a mean carcass weight of 250 kg (G0), 243 kg (G1) and 272 kg (G2). Carcasses were classified according to the EUROP system as R+2+ for G0, U−2+ for G1, and U2+ for G2. At 48 h postmortem, the Longissimus th. was dissected from the left half-carcasses and transported to the Meat Technology Centre. Subsequently, pH, L*, a*, b* and the percentages of moisture, fat, protein, and ash were determined. Finally, the extraction, derivatization, methylation, and chromatographic analysis of fatty acids present in the Longissimus th. were carried out. All statistical analyses were performed using R Commander for Windows, version 4.3.2. High oil supplementation (G2) increased significantly ash (G0: 1.10 ± 0.07 vs. G2: 1.16 ± 0.03, $P < 0.05$) and protein contents (G1: 21.40 ± 0.53 vs. G2: 22.06 ± 0.29, $P < 0.05$) and reduced intramuscular fat compared with G0 (G0: 1.95 ± 0.68 vs. G2: 1.00 ± 0.55; $P < 0.01$). Muscle $pH_{(24)}$ was higher in G2 but remained within acceptable technological ranges. Significant differences in trichromatic coordinates were observed between G2 and the G0 and G1 treatment group. L* value was significantly higher in G2 than in G1, with no appreciable differences compared with G0 (G1: 42.95 ± 3.87 vs. G2: 48.38 ± 5.12, $P < 0.05$). Similarly, a* value was higher in G2 than in G1, while no significant differences were detected between G0 and the supplemented groups (G1: 12.46 ± 0.85 vs. G2: 14.15 ± 1.08, $P < 0.01$). The value of b* was significantly higher in G2 compared with both G0 and G1 (G0: 13.71 ± 1.08 and G1: 14.09 ± 1.45 vs. G2: 16.56 ± 2.51, $P < 0.05$).Total PUFA content was significantly higher in G2 than in G0 (G0: 12.19 ± 2.42 vs. G2: 17.87 ± 4.72; $P < 0.05$), with increased n-3 PUFA, including EPA (G0: 0.40 ± 0.11 vs. G2: 0.80 ± 0.31, $P < 0.01$), DHA (G0: 0.12 ± 0.02 vs. G2: 0.19 ± 0.05, $P < 0.01$) and DPA (G0: 1.01 ± 0.20 vs. G2: 1.63 ± 0.46, $P < 0.05$). The PUFA/SFA ratio improved in G2 (G0: 0.26 ± 0.09 vs. G2: 0.39 ± 0.11, $P < 0.05$), while total SFA did not differ among treatments. C14:0 decreased in G2 (G0: 2.71 ± 0.61 vs. G2: 1.98 ± 0.19, $P < 0.01$). The n-6/n-3 ratio remained below recommended thresholds in all groups (G0: 3.93 ± 0.46, G1: 3.78 ± 0.50 and G2: 3.78 ± 0.30, n.s.). In conclusion, supplementation with 20 mL/animal/day of hydrolysed R. communis oilseed improve meat physicochemical quality. In general, meat colour improved significantly in G2, with higher L*, a* and b* values, suggesting enhanced oxidative stability associated with bioactive compounds naturally present in R. communis oilseed. Finally, the treatment resulted in a more favourable nutritional lipid profile through increased PUFA deposition, supporting its potential as a functional feed additive in Rubia Gallega veal production.

Immune-activating PhytoComplex solution reduces cryptosporidiosis severity in neonatal calves through Interleukin-18–mediated host defense

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Cryptosporidiosis, caused by Cryptosporidium parvum, is one of the most common causes of neonatal calf diarrhoea worldwide and leads to impaired growth, intestinal damage, and substantial economic losses. Due to the absence of effective vaccines or curative treatments for calves, nutritional strategies that enhance early-life immune readiness are of increasing scientific interest. A proprietary PhytoComplex solution (PC15, Selko US, Indianapolis) containing green tea, cinnamon, and pomegranate extracts has shown potential in a cryptosporidiosis mouse model to support innate immune activation, particularly via interleukin-18 (IL-18)–mediated antimicrobial peptide (AMP) production. Two randomised challenge trials involving male Holstein calves were conducted to investigate whether PC15 supplementation improves clinical outcomes, reduces oocyst shedding, and enhances innate immune responses in neonatal calves experimentally infected with C. parvum. In the first trial, fifteen calves were assigned to three groups: uninfected controls, infected controls, and infected calves receiving 25 mg PC15/kg BW (equivalent to 1333 mg/kg CMR). All infected calves received 5×10^5 C. parvum oocysts orally on day 3. Faecal oocyst shedding (qPCR), faecal scores, body weight, and intestinal IL-18 expression (day 14) were recorded. In the second trial, twenty-five calves were allocated to uninfected controls, infected controls, or infected calves receiving 3.75, 7.5, or 11.25 mg PC15/kg BW (200–600 mg/kg CMR). Study duration was 10 days. Methods matched the first trial. In both studies, infected control calves exhibited typical cryptosporidiosis symptoms, including diarrhoea, reduced body condition, and progressive oocyst shedding. In the first trial, PC15-supplemented calves showed no clinical signs of disease, gained 778 g/day more weight ($P < 0.0001$), and exhibited 99% lower oocyst shedding on day 14 ($P = 0.0013$) compared with infected controls. IL-18 expression was significantly elevated ($P < 0.0001$), and intestinal structure remained intact. In the second trial, PC15 produced clear dose-dependent effects: calves receiving the highest inclusion rate achieved 478 g/day greater weight gain ($P < 0.0001$) and up to a 201-fold reduction ($P = 0.009$) in oocyst shedding compared with infected controls. PC15 significantly reduced the severity of cryptosporidiosis across two independent challenge studies. The findings support an immune-mediated mode of action, whereby PC15 enhances IL-18-driven antimicrobial peptide production and strengthens intestinal defence mechanisms. These results indicate that PC15 may contribute meaningfully to early-life resilience against enteric parasitic infections in calves. Keywords: Cryptosporidiosis, neonatal calves, phytogenics, PhytoComplex solution, IL-18, intestinal immunity, oocyst shedding

Supplementing a Bacillus-based probiotic to mitigate frothy bloat in beef cattle receiving a high-concentrate diet

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Bacillus-based direct-fed microbials are proposed to modulate ruminal fermentation and microbial ecology, but their effects on physicochemical factors associated with frothy bloat are not fully understood. This experiment compared the incidence of frothy bloat, as well as ruminal, physiological, and performance responses of beef heifers receiving a bloat-provoking diet and supplemented with a Bacillus-based probiotic (BAC; Bacillus licheniformis + B. subtilis; BovacillusTM; Novonesis, Lyngby, Denmark). Sixteen ruminally-cannulated yearling Angus-influenced heifers were ranked by pre-trial body weight (BW) and assigned to 2 groups of 8 heifers each. Groups were enrolled in a 2 x 2 Latin square design containing 2 periods of 28 days, and a 14-day washout interval between periods. Groups were assigned to receive no BAC supplementation (CON; n = 16) or BAC supplementation at 3.0 g/heifer daily (n = 16) from day 0 to 28. During each period, heifers (n = 16/treatment) were housed in individual pens, and fed a sorghum-based bloat-provocative diet at 2% of their BW. Diet and treatments were individually fed to heifers, twice daily in equal proportions (0700 and 1600 h). Heifer unshrunk BW was recorded on days -3, -2, and -1 relative to the beginning of each period and averaged for initial BW. Final BW was calculated by averaging heifer BW on days 26, 27, and 28 of each period. Heifers were assessed for bloat score (0 to 5 scale, increasing according to bloat severity) 3 h after the morning feeding. Rumen fluid samples were collected via rumen cannula on days 0 and 28 of, immediately prior to (0 h) and at 3, 6, and 9 h relative to the morning feeding. Results obtained on day 0 were averaged and used as independent covariate in each respective analysis. No treatment differences were detected ($P \geq 0.45$) for heifer BW gain or gain:feed ratio. No treatment differences were detected ($P \geq 0.59$) for mean bloat score during the experiment (1.15 vs. 1.16 for BAC and CON heifers, respectively; SEM = 0.95) and rumen pH on day 28 (5.83 vs. 5.80 for BAC and CON heifers, respectively; SEM = 0.06). Heifers assigned to BAC had less ($P = 0.04$) rumen fluid viscosity (3.15 vs. 3.89 mPa.s.; SEM = 0.32) and less ($P = 0.05$) ruminal protozoa count on day 28 compared with CON heifers (8.53×10^5 vs. 1.02×10^4 cells/mL; SEM = 7.0×10^4). Heifers assigned to BAC had less ($P = 0.03$) valerate concentrations in the rumen fluid compared with CON heifers (4.41 vs. 5.21 mM, respectively; SEM = 0.55), whereas no other treatment effects were detected ($P \geq 0.14$) for concentrations of individual and total volatile fatty acids in the rumen fluid. In summary, supplementing BAC to beef heifers consuming a sorghum-based bloat-provocative diet decreased viscosity and protozoa population in the rumen fluid, but did not improve rumen fermentation responses nor mitigated the incidence of frothy bloat.

Effects of vitamin D_3 on cell viability, myogenic regulatory factors, and antioxidant responses in bovine skeletal muscle cells under heat stress

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Heat stress is a major environmental challenge that impairs skeletal muscle development and redox homeostasis in cattle. Vitamin D_3 (1,25-dihydroxyvitamin D_3) is known to regulate muscle metabolism and oxidative balance. However, its role in bovine skeletal muscle cells under heat stress remains unclear. This study aimed to evaluate the effects of vitamin D_3 on cell viability, myogenic regulatory factors, antioxidant gene expression, and heat shock proteins in heat-stressed bovine skeletal muscle-derived cells (BSMCs). BSMCs isolated from Korean native cattle (Hanwoo) were treated with vitamin D_3 (0, 1, 10, and 100 nM) and exposed to heat stress at 41 °C for 48 h. Cell viability was evaluated using a CCK-8 assay in a separate experiment. For gene expression analysis, cells were exposed to heat stress (41 °C) for 48 h during mid-differentiation (d 2–4), and samples were collected immediately after heat exposure (d 4). The expression of myogenic regulatory factors (Myf5, MyoD, MyoG, Myf6), antioxidant enzymes (GPX1, GPX4, SOD1, SOD2, NRF2), and heat shock proteins (HSPB1 and HSP70) was analyzed by qPCR. Data were analyzed using the MIXED procedure of SAS, and significance was declared at $p < 0.05$. Heat stress significantly reduced cell viability ($p < 0.01$). Vitamin D_3 treatment improved cell viability under heat stress. The 1 nM group restored viability to a level comparable to the normothermic control, whereas the 10 and 100 nM groups did not fully restore viability to the control level. Heat stress significantly increased HSPB1 ($p < 0.01$) and HSP70 ($p < 0.05$), and this increase remained high across all heat-exposed groups. Heat exposure altered myogenic regulatory factors, with significant changes observed in Myf5 and Myf6 ($p < 0.01$), whereas MyoD and MyoG were not significantly different. Antioxidant-related genes showed heat-responsive changes. GPX1 and SOD1 were significantly increased under heat stress ($p < 0.01$). SOD2 expression was increased by heat stress and decreased with increasing vitamin D_3 concentration ($p < 0.01$). NRF2 was significantly increased only in the 100 nM group under heat stress ($p < 0.05$), whereas GPX4 was not significantly different ($p = 0.0625$). In conclusion, vitamin D_3 improved cell viability under heat stress and modulated myogenic and antioxidant gene expression, whereas heat shock protein induction remained robust under heat exposure. These findings indicate the potential of vitamin D_3 as a nutritional strategy to support muscle cellular stability during thermal stress in cattle.

Targeted cis-9, trans-11 CLA bioconversion in in vitro rumen fermentation catalyzed by Bifidobacterium-based probiotic and postbiotic supplementation

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Conjugated linoleic acid (CLA) is a bioactive lipid widely consumed as a dietary supplement due to its reported health promoting properties. Commercial CLA is typically derived from plant oils via chemical synthesis; however, this process generates a heterogenous isomer mixture, reducing biological efficacy due to poor isomer specificity. Microbial biosynthesis offers a superior alternative, as microbial enzymes preferentially convert linoleic acid (LA) into a single CLA isomer, most commonly cis-9, trans-11 isomer. In this study, Bifidobacterium breve JKL2022, a potent producer of cis-9, trans-11 CLA, was investigated for potential applications beyond conventional probiotic use. Probiotic and postbiotic preparations of JKL2022 were evaluated as biocatalysts for CLA production in two systems: in vitro vegetable oil fermentation and in vitro rumen fermentation. Under a controlled one-step in vitro digestion of grapeseed oil with CRL lipase and JKL2022 linoleic acid isomerase, preferential conversion to cis-9, trans-11 CLA was achieved at high efficiency (87.71 ± 1.45% – 91.27 ± 0.79%). Efficient conversion of conjugated linolenic acid (CLnA) from perilla seed oil was also observed, highlighting the feasibility of valorizing low-cost plant oils into high-value bioactive lipids. Under in vitro rumen fermentation conditions, supplementation of either probiotics or postbiotics preparation of JKL2022 (10^{10} CFU/mL) resulted in early CLA production. Addition of 0.5 – 1.0 mg/mL of LA lead to detectable cis-9, trans-11 CLA within the first 3 h of fermentation, catalyzed by probiotic (21.76 ± 3.33 µg/mL) and postbiotic (16.84 ± 2.08 µg/mL) preparations. Further fermentation shifted the lipid profile from CLA toward vaccenic acid (C18:1), consistent with rumen microbial biohydrogenation. Importantly, early CLA production may enhance mammary uptake of CLA and vaccenic acid, potentially increasing milk CLA content. Overall, B. breve JKL2022 exhibits a unique capacity for highly specific CLA biosynthesis in both food and rumen-simulated systems. These findings support its development as a probiotic or postbiotic strategy for targeted bioactive lipid production, with potential application in functional feeds, dairy quality enhancement, and sustainable lipid bioprocessing.

The effect of lowering the crude protein of dairy diets with the addition of either a molasses based liquid feed (Regumix) alone or with Regumix + methionine supplementation on production performance and efficiency

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Excess dietary protein increases urinary and faecal urea excretion, contributing to environmental load. Providing rapidly fermentable carbohydrates, such as sugars, can improve ruminal nitrogen capture and reduce nitrogen losses with a recent trial showing a 7.6% reduction in urinary nitrogen when feeding Regumix, a 27% protein molasses-based liquid feed. Research on sugars in lower-CP diets and their effects on performance and health remains limited. Methionine is typically the first limiting amino acid in UK dairy diets, and overfeeding CP to supply essential amino acids is inefficient. Targeted amino acid supplementation offers a more sustainable approach amid pressure to cut carbon, nitrogen, and soya use. This study aimed to assess whether the nitrogen efficiency benefits of fermentable carbohydrates persist under reduced CP diets and whether methionine supplementation offers additional advantages on protein efficiency, milk production, and environmental sustainability in dairy cows. A continuous 11-week experiment was conducted with 42 lactating Holstein cows, including a 2-week covariate period followed by 9 weeks of measurement. Cows were assigned to one of three diets—Control (FD), Regumix (LF), or Regumix + MetaSmart methionine supplement (PLF)—with CP levels of 17.5%, 16.3% and 16.1%, respectively, and 14 cows per treatment. Blocking was based on covariate milk yield, stage of lactation and parity; cows were >35 L/day yield, targeted at 40 L, and 100–140 DIM at study start. Diets consisted of grass silage, maize silage, concentrate blend and molasses-based liquid feeds, fed ad libitum as a TMR via Calan gates. Diets were formulated to be isoenergetic by adjusting the blend and adding 1.4 kg of each liquid feed to LF and PLF. Measurements included daily DMI and weekly milk yield, milk composition, liveweight and BCS. Data was analysed using the Mixed procedure of SAS® and a model testing fixed effects of treatment, week, their interaction, and the random effect of cow within block, using covariate measurements and blocking variables (parity, days in milk) as fixed effects. Results found no statistical difference in milk yield across the 3 treatments despite lower dietary protein levels. When dietary CP was analysed and calculated, the actual CP feeding levels were 17.4%, 15.4% and 15.4% for the FD, LF and PLF respectively. Cows on the PLF diet exhibited higher milk protein percentages and improved BCS in late lactation, showing greater resilience and indicating better energy balance and metabolic health. Total Protein Efficiency (TPE) was calculated as follows TPE = (milk yield×milk protein %)/(DMI×CP%), and showed a 17% improvement with the PLF diet, demonstrating more effective conversion of dietary protein into saleable milk protein. Lower milk urea concentrations in both treatment groups suggested enhanced nitrogen metabolism. In conclusion, precision liquid feeding offers a viable and sustainable alternative to traditional high CP diets enhancing protein efficiency and supporting cow health through maintenance of BCS through lactation. By reducing the CP feeding level by 1.4% in a ration, this could, for example, equate to a removal of around 300g of high-protein soybean meal per cow per day, equating to a potential saving of over 185,000 tonnes annually across the UK dairy herd helping meet environmental goals in the industry.

Dietary Polyphenols Modulate Milk Production and Udder Inflammation in Commercial Dairy Herds

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The use of polyphenols in dairy cows nutrition has gained attention due to their antioxidant and anti-inflammatory properties and their potential to modulate the ruminal microbiota, favoring metabolic efficiency regarding nutrient utilization and mammary gland health. These bioactive compounds have been investigated as sustainable alternatives capable of improving productive performance and reducing losses associated with subclinical mastitis. Considering such benefits, it becomes relevant to study its application in commercial herds and to determine the effects of the polyphenolic bioactive on milk production and udder health, at different stages of lactation and classes of somatic cell count. Therefore, this study was conducted on 10 commercial dairy farms in the State of São Paulo, Brazil, totaling 429 lactating cows, whose objective was to evaluate the effect of the polyphenolic compound extracted from sugarcane (Polygain™, The Product Makers, Australia) on milk production and mammary gland health. Supplementation was provided for 28 days, at 50 g/animal/day dosage, incorporated into the diet and without any changes in feeding or milking protocols. Individual data on milk yield (kg/cow/day) and somatic cell count (SC $\times 10^3$/mL) were collected on days 0 (D0) and 28 (D28). SCC analyses were performed in a reference laboratory, and the values were log-transformed for statistical analysis. For the purpose of SCC classification, cows were categorized into three categories: class 1 (≤200 thousand cells/mL), class 2 (201–500 thousand cells/mL), and class 3 (>501 thousand cells/mL). Lactation stage was also categorized into three phases: early (≤100 days), mid (101–200 days), and late (201–305 days). Data normality was verified by the Shapiro-Wilk test. Comparisons between D0 and D28 were performed using the paired Wilcoxon and KruskalWallis tests, adopting a statistical significance level of 5%. The polyphenol supplementation showed specific effects according to lactation stage and SCC class. For cows in class 1 and in early lactation, a significant increase in milk yield was observed, from 41.5 to 42.8 kg/day (P=0.0133), although this effect is more associated with the production peak of the period than with the bioactive. Regarding SCC, a significant reduction was observed only in animals in class 3. For the early stage, SCC decreased from 1,891 to 139 thousand cells/mL (P=0.0078), while for late lactation it decreased from 2,551 to 1,835 thousand cells/mL (P=0.0234), indicating a better response of the product in cows with more pronounced inflammation. Supplementation with polyphenols derived from sugarcane extract demonstrated potential to improve mammary gland health indicators, especially in cows with elevated SCC, in which expressive reductions were observed. The effect on milk yield was limited and mainly influenced by lactation stage. These results suggest that the use of polyphenolic compounds may be a complementary strategy to support udder health, particularly in animals with greater inflammatory challenge, contributing to more efficient and sustainable production systems.

Effect of a phytogenic ingredient mixture on rumen degradability of soybean meal protein

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Improving nitrogen (N) efficiency is of utmost importance for farmers to lower their N emissions while maximizing farm profits. Phytogenic ingredients (PHY) can be used to decrease rumen degradability of dietary protein, thereby nutritionally optimizing cattle diets in an economic way and allowing farmers to comply working within tighter environmental constraints. An in vitro experiment was designed to evaluate the effect of Nsure, a mixture of PHY, on rumen degradability (DM and CP), NH3-N accumulation and fermentability (CH4 and volatile fatty acids; VFA) of soybean meal (SM). Therefore, 500 mg, consisting of 475 mg SM (449 g CP/kg), Nsure (0, 3.125, 6.25, 12.5 or 25 mg) and limestone as filler, was placed into sealed nylon bags (pore size 37 µm) and incubated in triplicate in sealed CO2-flushed 250-ml flasks for 24h in a shaking incubator with 50 ml CO2-saturated phosphate/bicarbonate-buffer (0.8 v/v) and inoculum (0.2 v/v) derived from 3 rumen fistulated dairy cows. A linear decrease in degradability of SM was observed with increasing Nsure inclusion for DM (70.0, 69.5, 68.1, 67.2 and 65.3 % of DM0h, resp.; -0.93% per % Nsure, SE=0.374, R^2=0.32, P=0.027) and CP (49.6, 52.1, 49.4, 43.2 and 37.4 % of CP0h, resp.; -2.90% per % Nsure, SE=0.698, R^2=0.57, P=0.001). Protection against ruminal degradation seemed specific to the protein fraction of SM, as there was no linear effect (P=0.684) on the degradability of the non-CP DM fraction (83.9, 81.5, 80.6, 82.7 and 83.2 % of non-CP DM0h, resp.). Furthermore, NH3-N accumulation linearly decreased with increasing Nsure inclusion (311, 289, 282, 230 and 187 mg/L, resp.; -25.1 mg/L per % Nsure, SE=4.99, R^2=0.66, P<0.001). Also absolute CH4 and total VFA was linearly impacted (P=0.006), presumably because less degradable CP was available for microbial fermentation, without affecting relative CH4 production (mol/mol CH4/VFA; P=0.721). Molar proportions of iso-butyrate and -valerate, being indicators of CP degradation, were linearly decreasing as well (P<0.001), underlining the protective effect against ruminal protein breakdown. Further in vivo research is needed to validate the effect of Nsure on N efficiency in real life conditions.

Optimizing amino acid supply in early lactation cows improves production performance in commercial farm conditions

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Optimizing amino acid supply, particularly methionine, during the early stages of lactation is a key strategy to enhance metabolic efficiency and milk production in high-yielding dairy cows. This study aimed at evaluating the effects of rumen-protected methionine (RP-Met) supplementation on production performances of early and peak lactating cows. A total of 140 Holstein cows from a German commercial farm were divided at calving into two experimental groups fed either a basal diet alone (CON) or supplemented with 15 g/day/cow of RP-Met (RPM, Mepron®, 85% DL-methionine, Evonik Industries, Germany). The supplementation lasted 14 weeks, but performance were evaluated for another 4 weeks to assess the carry-over effect of RP-Met. Individual milk yield (MY) was registered daily with an automated milking system (AMS) and milk composition were assessed monthly via the official milk recordings national agency. Statistical effects were analyzed based on a linear model using treatment and period (week) and their interaction as fixed effects. Least square means were compared using Tukey's test. Significance was declared when $P \leq 0.05$ and tendency with $0.05 < P \leq 0.10$. Milk yield significantly increased by +1.7 kg/day during the supplementation period with RPM in comparison to CON ($P<0.001$), while milk fat and protein content were not affected. Energy corrected milk (ECM) was significantly higher for the RPM group ($P<0.001$; 34.7 and 33.1 kg/day for RPM and CON, respectively) mainly driven by the higher milk fat yield ($P=0.017$; 1.37 and 1.30 kg/day for RPM and CON, respectively) and protein yield ($P=0.043$; 1.20 and 1.16 kg/day for RPM and CON, respectively). After finishing RPM supplementation, MY remained significantly higher for RPM in comparison to CON ($P<0.001$; 33.1 and 31.9 kg/day, respectively). The significant carry-over effect on MY after RP-Met supplementation highlights the importance of optimal methionine supply in improving the lactation persistency in high producing dairy cows. These results confirm the value of RP-Met as a practical tool for amino acid balancing and metabolic optimization in modern dairy production systems.

Microalgae supplementation enhances omega-3 PUFA in lamb meat and its consumption improves blood fatty acid status in consumers

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Long-chain omega-3 (n-3) polyunsaturated fatty acids (FA), including eicosapentaenoic acid (C20:5 n-3; EPA) and docosahexaenoic acid (C22:6 n-3; DHA), are essential for optimal human health and well-being. Traditionally, n-3 in lamb is derived from grass-based diets, but alternative strategies are needed to further enhance its nutritional profile. Microalgae finishing also offers sustainability benefits, including the potential to reduce methane emissions from livestock. The aim of this study was to investigate the impact of consuming microalgae finished lamb meat, compared to a control, on blood FA response and cardiovascular risk factors among healthy consumers. Lambs were supplemented with microalgae supplemented (1.62% DM) or control diets (0% DM) for 11 weeks until slaughter. Healthy participants then consumed two portions per week of these lambs in a six-week, single-blinded, dietary intervention. Blood samples were taken at baseline (week 0), mid-way (week 3) and post-intervention (week 6) for FA and cholesterol analysis. Weight, body mass index (BMI) and blood pressure measurements were also taken. FAs from blood plasma, plasma phospholipids and red blood cells were extracted using a chloroform: methanol method. Fatty acid methyl esters (FAMEs) were analysed by GC-FID. Repeated measures ANCOVA, with baseline values as covariates, was used to evaluate the effects of lamb treatment (microalgae-finished vs. control) over time, including treatment, time, and treatment X time interactions ($p < 0.05$). Microalgae finishing increased the n-3 content of the lamb, and consumption of this meat led to detectable changes in blood FA profiles. Changes in cholesterol fractions were observed, while BMI and blood pressure remained unchanged. Regular consumption of microalgae-finished lamb, as part of a balanced diet, increases blood n-3 FA levels, which is associated with improved health and well-being.

Grape pomace silage in the finishing diet of beef steers

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Vitis vinifera L. is one of the most widely cultivated fruit crops, with an annual production exceeding 67 million tons and generating up to 200 kg of solid waste per 750 liters of wine. Approximately 60% of these by-products is grape pomace, a mixture of skins, seeds, stems, wastewater, and lees. Incorporating grape pomace into animal diets is a promising strategy to reduce feed costs, disposal expenses, and competition for food resources between humans and livestock. This study evaluated the inclusion of grape pomace silage (GPS) at two different inclusion levels in the finishing diet of beef steers and its effects on body weight, blood biochemical and oxidative profiles, and meat quality during dry aging. Finally, this study aimed to compare the cradle-to-farm gate environmental sustainability of two different inclusion levels of GPS. A total of 48 crossbred Holstein × Belgian Blue steers were enrolled in the trial. The experiment was conducted in three replicates, spaced two weeks apart. Within each replicate, the 16 animals were randomly assigned to one of three dietary treatments: a control group (C) receiving 150 g/kg DM of wheat straw, a low grape pomace group (LGP) receiving 50 g/kg DM of grape pomace replacing straw, and a high grape pomace group (HGP) receiving 100 g/kg DM of grape pomace replacing straw. The feeding period lasted 60 days prior to slaughter. Body weights and blood samples were collected at the start of the feeding trial and after 60 days. Blood samples were analyzed to assess clinical biochemical and oxidative profiles. 24 h after slaughter bone-in loins from the thirteenth thoracic vertebrae to the sixth lumbar vertebrae were collected from the left side of each carcass and subjected to dry aging for 42 days. At each experimental time point (0, 14, 21, 28, 35, and 42 days), a 30 mm steak was cut from each loin for the analyses. Meat samples were evaluated for instrumental color, water-holding capacity, cooking loss, and oxidative profile, including both oxidation products and antioxidant potential (FRAP assay). Life cycle assessment (LCA) was used for environmental impact assessment. The functional unit was 1 kg of live weight gain. The studied environmental profile was applied to three categories of impact: global warming potential (GWP, kg CO2 -eq), water depletion (WD, m3 eq), and abiotic depletion of fossil fuels (ADP fuel, MJ), by using the SimaPro 10.1 PhD, PRé Consultants. Data were analyzed using SAS statistical software. Normality and homogeneity of variance were checked prior to analysis. An ANOVA (GLM) was then applied, considering diet, time, and their interaction as fixed effects. Preliminary results showed no significant effect of diet on blood clinical biochemical or oxidative profiles. Similarly, meat quality parameters were not significantly affected by diet, with the exception of TBARS. Notably, meat from animals fed GPS showed significantly lower TBARS concentrations during the dry aging period ($P<0.001$). These preliminary results suggest that GPS can be incorporated into finishing diets without compromising animal health, growth performance, or meat quality of beef steers. LCA results showed that LGP has the lowest GWP (1.8 kg CO2 eq,) WD (3.81 m3 eq). and ADP fuel (2.13) depletion compared with C and HGP. Moreover, this study also used the ratio between product value and environmental impact as an indicator of eco-efficiency: results highlighted that the eco-efficiency for main categories is most effective with LGP.

Multivariate relationships between carcass, muscle, and beef eating quality traits in Limousine cull cows

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Adopting a grading scheme inspired by the Meat Standards Australia (MSA) system is considered an effective way to strengthen consumer confidence in beef across Europe. However, differences between Europe and Australia in cattle breeds, production systems, and consumer dietary habits require a re-examination of the links among carcass traits, muscle traits, and beef eating qualities. In France, cull cows constitute a major beef source, with Limousine cattle being one of the predominant breeds. Accordingly, this study evaluated 102 French Limousine cull cows (mean age 125.8 months) to investigate the relationships among carcass, muscles, and beef eating quality traits, and to explore the predictive value of key biological indicators for beef palatability. A comprehensive dataset integrating carcass grading traits assessed using the EUROP grid and the MSA protocol, muscle laboratory measurements, and consumer sensory score of beef was analyzed using correlation analysis, principal component analysis (PCA), and linear mixed-effects modelling. Carcass yield-related traits (carcass weight, conformation, and eye muscle area) were highly intercorrelated ($r > 0.8$) and moderately associated with consumer-evaluated eating quality. In contrast, indicators of intramuscular fat deposition (marbling and intramuscular fat content) showed no direct correlation with eating quality. PCA revealed a clear structural opposition between eating quality traits and compression force, highlighting compression force as a more informative structural indicator than Warner–Bratzler shear force (WBSF) in beef from older Limousine cull cows. When classifying carcasses based on the MSA grades, high consumer-graded samples clustered with multiple carcass traits related to yield and fat deposition, whereas low-graded samples were primarily associated with older age, higher compression force, elevated pH, and greater SFA content. Linear mixed-effects models demonstrated that marbling showed minimal contribution to eating quality prediction across muscles (marginal $R2 \leq 0.01$). In contrast, traits related to muscle development, including age, carcass conformation, and compression force, played a dominant role in determining eating quality ($P < 0.05$). Model performance differed between MSA grade groups. High-MSA grade samples exhibited more comprehensive biological features and achieved higher predictive accuracy. However, low-MSA grade samples showed reduced model performance, likely due to narrower sensory variation and higher trait homogenization. These findings indicate that predictors of beef eating quality depend on animal type and age. In older cull cows, predictors of eating quality are likely to be more specific, particularly those related to muscle structure and maturity, at least in part. Overall, these findings provide practical guidance for the beef industry in predicting beef eating quality and improving grading systems for cull cows.

The effect of post-slaughter time on marbling scores in beef carcasses

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Marbling is used throughout the beef industry to describe the visual fat characteristics of a specific cut, commonly the rib eye. Establishing a reliable and accurate grading method is imperative as international markets pay a premium for highly marbled products. At present, the standard practice for grading marbling involves the use of trained professionals who visually score the cuts, referencing the AUS-MEAT and MSA scoring system. AUS-MEAT scores range from 0-9, considering the amount of fat, whereas the MSA system scores range from 100 – 1190, which additionally takes into account the fineness and distribution of fat. However, recent imaging technologies such as the Q-FOM camera have been developed, allowing a much higher level of objective measurement and accuracy. Marbling scores are expected to increase over time, but to what extent and how rapidly remains undetermined. This is potentially beneficial to the meat processing plant as graded product may not be expressing the potential maximal marble score. Therefore, the objective of this research is to establish the optimal grading time post-slaughter, using Q-FOM grading technology. It was hypothesised that marble scores from both grain and grass-fed cattle will not increase after 24h and up to 96h post-slaughter. It is also hypothesised that grain-fed cattle will have greater marble scores compared to grass-fed cattle. A total of 120 carcasses (n = 60 grass-fed, n = 60 grain-fed) were graded visually and using the Q-FOM, from 24 h post-slaughter, up to 96 h. These 5 separate timepoints were taken from 6 individual chillers at the collaborating processing plant, and carcasses ranged from 210-980 MSA marble score. Samples from each animal were chemically analysed in a laboratory to determine intramuscular fat percentage (IMF%). Statistical analysis was conducted in RStudio, using a linear mixed effects model to test the interactions between IMF%, time and diet on marbling score. This helped to predict the change in marbling over time and how this differs between diets. It was determined that marble scores increase up until 47 hours post-slaughter, with no further increase in marble score beyond this time point. Between 24 and 36 hours, the exact rate at which marbling develops needs further investigation. As hypothesised, the grain-fed animals did produce greater marble scores compared to the grass-fed. Current practice of delaying the grading of grain-fed beef is maximising marble scores; grass-fed animals are not typically graded following the same protocols. These carcasses are graded at a maximum of 24 hours post-slaughter, which may not be sufficient time for the optimal marble score to be achieved. By extending this time to grading, there will be operating costs associated, as well as storage space, however this may be plausible for processing facilities. Further research is required to determine this potential return on investment.

Physicochemical quality differentiation of European beef cuts by retail price category, sex, and region reared

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European beef production is highly fragmented, characterised by heterogeneous genetics, feeding regimes, and processing conditions that influence meat quality. Unlike unified carcass grading systems, the European beef market relies heavily on retail cut-based pricing, yet the relationship of these commercial price categories and physicochemical traits remains largely unquantified. This study tested whether retail price-based cut categorisation differentiates physicochemical quality, with sex and European region reared providing independent variation for market segmentation. Commercial beef cuts (n=83) were classified into Low (n=22; topside, brisket), Medium (n=39; rump, knuckle, flat iron), and High (n=22; tenderloin, picanha, cube roll, striploin) Price categories. Samples were sourced from Western (n=17; France, Holland, Ireland, Spain), Central (n=34; Austria, Czech Republic, Hungary, Slovakia), and Eastern (n=32; Poland, Latvia) Europe, and stratified by Sex (male, female). Assessed traits included colour, pH, cooking loss, water holding capacity (WHC), shear force (SF), and proximate composition. The obtained data were analysed using the GLM procedure of SAS. The model included the fixed effects of Price, Cut nested within Price, Sex, Region reared, and their 2-way interactions. The Price effect was tested using Cut nested within Price as the error term, whereas the other fixed effects were tested on the residual error. Model R^2 ranged from 0.27 to 0.70, indicating robust model fit for key traits. Price category significantly affected SF (F=7.04, P=0.027). The nested contrast confirmed a systematic tenderisation gradient scaling directly with price: Low (57.2 N), Medium (44.4 N), and High (23.3 N) (all pairwise P<0.05). Conversely, the Cut (Price) nested effect dominated compositional and colour traits (water F=9.94, protein F=4.03, fat F=7.08, collagen F=7.44, WHC F=6.62; P<0.01), reflecting how muscle-specific physiology drives biochemical composition within broader commercial price brackets. Sex independently influenced quality and functionality trade-offs. Compared to males, females exhibited higher fat (4.90% vs. 3.57%; P=0.038), elevated nutrition scores (129 vs. 117 kcal/g; P=0.026), greater fat-to-protein ratios (0.23 vs. 0.17; P=0.046), and significantly more tender meat (lower SF: 35.6 vs. 47.6 N; P=0.007). However, female beef displayed slightly lower WHC (40.7% vs. 41.2%; P=0.037). Region reared systematically shaped appearance and hydration profiles. Western beef presented superior lightness (L* 35.4 vs. 32.3 Central and 32.3 Eastern; P=0.003); chroma (C* 26.2 vs. 24.6 Central and 23.6 Eastern; P=0.036), alongside the highest salt content (1.10% vs. 0.76% Central and 0.82% Eastern; P=0.002). Eastern beef retained the highest overall water content (69.0% vs. 68.9% Western and 68.3% Central; P=0.012) but the lowest WHC (40.2% vs. 41.4% Western and 41.0% Central; P=0.005), suggesting fundamental variations in post-mortem chilling, aging, or processing practices across regional supply chains. All two-way interactions were non-significant (P>0.05), proving these three main effects operate independently. In conclusion, retail price categorisation serves as a reliable predictor of tenderness in the European beef market, successfully validated when isolating cut effects. Furthermore, sex differentiates energy density and tenderness, while rearing region shows distinct processing and hydration signatures. Integrating these independent parameters provides a comprehensive, scientifically validated framework for quality assessment, product valuation, and targeted consumer communication in the meat industry.

REIMS-based metabolic fingerprinting discriminates animal categories across muscle types, and ageing regimes in beef loin

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Meat ageing is a complex biochemical process influenced by multiple biological and processing factors. Rapid evaporative ionization mass spectrometry (REIMS) is a minimally invasive analytical technique with direct measurement times <10 seconds, that has shown potential for monitoring quality changes during meat ageing. The ability of REIMS to discriminate meat samples from different backgrounds and ageing conditions remains underexplored. We hypothesised that REIMS can discriminate beef samples across animal categories, muscle types, and ageing conditions, and that its performance depends on the magnitude of biochemical changes induced during ageing. In this study, REIMS was applied to a multiclass dataset including aged meat samples from multiple animal categories (adult cattle, yearling cattle, heifers, and steers) across three beef cuts (loin, chuck, and topside). Loin samples from different categories were further subjected to either wet-ageing to test the effects of ageing time (0, 5, and 21 d) or to ageing regimes (unaged, in-bag dry ageing, stepwise ageing) for 28 d. Stepwise ageing refers to wet-ageing for 14 d followed by in-bag dry-ageing for 14 d. Multivariate classification was performed using orthogonal partial least squares – hierarchical discriminant analysis (OPLS-HDA), and multivariate modelling with unbiased variable selection in R (MUVR) was applied for model validation. REIMS fingerprinting exhibited robust discrimination of muscle cuts across adult and yearling beef with an accuracy of 80.5%, indicating that muscle-specific structural and metabolic characteristics represented the dominant source of variation compared with animal category. Discrimination of wet-ageing time of beef loins achieved an overall accuracy of 70.8%, with a clear difference in performance between animal categories. Pronounced class separation was observed in 21 d aged samples from adult cattle compared with unaged samples and aged for 5 d. In contrast, loin samples from yearling calves showed no clear discrimination in REIMS fingerprints across ageing time. In-bag dry-ageing was the primary factor distinguishing between ageing regimes, producing the greatest cluster separation from stepwise-aged and unaged samples, which partially overlapped. However, beef from heifers and steers could not be reliably distinguished under either dry or stepwise ageing, with a classification accuracy <50%. Overall, these results support the potential of REIMS as a rapid tool for discriminating animal age and muscle types, and evaluation of changes during ageing processes.

Growth performance in Chianina cattle: a 10-year database comparison between PGI production scheme and EU mandatory beef labelling system

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The production of the native Italian beef cattle breed Chianina is characterized by the coexistence of two management systems. The first is the mandatory Regulation (EC) No. 1760/2000 labeling system (ETQ-Ch), generally adopted by specialized fattening centers and characterized by standardized rations and greater use of non-local feed ingredients. The second is the PGI "Vitellone Bianco dell'Appennino Centrale" system (PGI-Ch), a voluntary scheme which imposes stricter requirements on geographical area, animal genealogy and feeding practices to strengthen the link between meat characteristics and territory. Quantitative evidence on how these different management strategies affect animal growth performance remains limited, despite their practical relevance for farm management. Therefore, we hypothesized that the PGI-Ch and ETQ-Ch production systems would differ in animal growth performance. The study was conducted using data obtained from the Italian National Cattle Database (BDN, Teramo). Male cattle recorded as Chianina in the BDN, aged between 600 and 750 days and slaughtered between 2012 and 2022 at the Perugia Meat Slaughtering Centre were included. Age at slaughter, carcass weight and average daily carcass gain (ADG) were evaluated. Animals with incomplete records for any of the analysed variables were excluded. The dataset comprised PGI-Ch (n = 17,415) and ETQ-Ch (n = 3,557) animals, for a total of 20,972 records. Differences between the systems were analysed using a general linear model including the fixed effect of the identification system (PGI-Ch vs ETQ-Ch). The relationship between age at slaughter and ADG was evaluated using simple linear regression. Normality and homoscedasticity assumptions were verified through residual analysis and variance tests. Outliers were identified through graphical inspection of residuals and evaluated based on biological plausibility. Results were considered significant when $P \leq 0.05$ and a tendency when $0.05 < P \leq 0.10$. Statistical analyses were performed using SAS® 9.4. Average slaughter age was 649.82 ± 74.62 days (SD) and 623.11 ± 86.71 days (SD) for PGI-Ch and ETQ-Ch, respectively, although the difference was not statistically significant. Average carcass weight was 480.24 ± 75.54 kg (SD) for PGI-Ch and 477.84 ± 72.19 kg (SD) for ETQ-Ch, showing a tendency towards heavier carcasses in the PGI-Ch group ($P = 0.07$). The ADG was higher in ETQ-Ch animals than in PGI-Ch animals (0.774 vs. 0.745 kg/day) ($P < 0.001$). This difference may reflect the feeding strategies adopted in ETQ-Ch, which often involve higher energy density. Moreover, this result may partly reflect genetic selection and the search for phenotypic standardization in the Chianina breed over time, contributing to more homogeneous production performance even among non-PGI animals. For both groups, a negative relationship was observed between age at slaughter and ADG, consistent with the physiological growth of the animals ($R^2 = 0.32$). In conclusion, the results suggest that animal performance parameters are influenced by the rearing system, with higher ADG for ETQ-Ch animals. Further research integrating farming management (feed ingredient origin, environmental conditions, etc.) and meat quality traits is needed to define sustainable production strategies while supporting the valorisation of native breeds and typical products.

When beef turns brown: a cross-country comparison of consumer preferences in Germany and USA

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In the USA, 197 million kg of beef is wasted annually due to discoloration at retail. To reach the Sustainable Development Goal (SDG) 12.3 of halving per capita food waste by 2030, an understanding of consumer preference for beef color is needed to tailor market strategies. Our research compares consumers' willingness-to-pay (WTP) for beef strip loin (longissimus lumborum) steak stored for up to 9 days under retail conditions. Photos of steaks during retail display at days 0, 4, 7, and 9 were incorporated into a discrete choice experiment including 4 levels of real market country-specific prices, and a sale sticker (30% off). Consumers in Colorado, USA (n=297; June – August 2023) and Lower Saxony, Germany (n=154; September – December 2025) completed the choice task programmed in Qualtrics running on an iPad. Choice data were analysed using multinomial logit models; price was a fixed effect variable and all other attributes were random variables. Discolored, i.e. steaks stored 4 – 9 days, were heavily discounted by consumers compared to day 0. USA consumers were more sensitive to price and discoloration than German consumers. Differences in German WTP between day 7 and day 9 were minimal, whereas USA estimates show a 100% decrease in their WTP from day 7 to day 9. Differences across gender were similar across countries, with females being more sensitive and discounting discolored steaks almost twice that of men. Although sale stickers on meat could be perceived as signaling reduced intrinsic quality, the sale sticker in our study had a positive coefficient across all models regardless of gender and/or country, and as such was perceived positively. This is most likely due to the perceived utility gains of saving money. However, according to the models' results, a 30% discount would not be sufficient for enticing consumers to purchase discolored steaks, even after day 4. We initially anticipated that USA consumers who are more familiar with beef steaks are less likely to discount discolored steaks; however, our results show otherwise. Although BBQ culture is growing in Germany, beef steak is considered a luxury cut and often consumed out of home; the reduced familiarity with a raw product may explain their reduced sensitivity to discoloration. Due to current consumer preferences based on our WTP estimates, technological innovation, e.g. packaging or freezing, strategies to improve color stability as well as educational marketing measures directed at meat color formation are required to reduce beef waste at retail. Shorter term mitigation measures could include color sorting in retail counters, to hinder the consumer's ability to compare across-product color.

Can bone mineral density by dual-energy X-ray absorptiometry predict age, shear force, and collagen in cull cows' carcasses?

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Cull cow carcasses often show high variability in eating quality, largely driven by maturity-related changes in connective tissue and their interaction with carcass composition. Dual-energy X-ray absorptiometry (DEXA) has been increasingly explored in livestock to predict carcass composition, and its bone outputs may also provide objective maturity-related information. We hypothesised that bone mineral density (BMD) measured by medical DEXA could predict chronological age, collagen traits, and tenderness in cull cows, particularly when combined with hot carcass weight (HCW). The objective was to evaluate the predictive capacity of regional and whole-carcass BMD for age (months), collagen content (mg/g), collagen solubility (%) and Warner–Bratzler shear force (WBSF, kgf) at 0 and 14 d post-mortem (SF0, SF14). Twenty-one Nellore cull cow carcasses (92.9 ± 35.5 months; 132.3 ± 19.0 kg) were used. The carcasses were chilled (4 °C, 24 h), and then the right side was divided into five anatomical regions (R1: longitudinal cut after the second rib; R2: longitudinal cut after the eighth rib; R3: longitudinal cut after the last rib; R4: longitudinal cut after the sacrum; and R5: remaining caudal section). Each region was DEXA scanned, and regional BMD (g/cm²), bone mineral content (BMC, g), and area (cm²) were recorded. Whole-carcass BMD was calculated as ΣBMC/ΣArea across the five regions. Collagen content and collagen solubility were determined by hydroxyproline-based assays, and WBSF was measured on longissimus lumborum samples. Data were analysed using SAS software (PROC GLM), individually testing regional BMD variables and their interactions with HCW. Models using only BMD explained a moderate proportion of variation in age ($R^2 \leq 0.30$, RMSE ≤ 34.8 months). Prediction improved when HCW was included ($R^2 \geq 0.71$, RMSE ≤ 22.0 months), and interaction models provided the best fit ($R^2 = 0.79$, RMSE = 17.8 months for BMD2×HCW) for age. Collagen content was not predicted by any models. In contrast, interaction models showed moderate explanatory power for collagen solubility and SF14, with the best being BMD5×HCW ($R^2 = 0.47$ and 0.55, RMSE = 1.6 % and 1.0 kgf, respectively). Single BMD models did not predict SF0 or SF14, whereas inclusion of HCW improved prediction for SF14. These results partly support the hypothesis that BMD has limited ability to predict age in cull cows, likely reflecting a plateau in bone mineralisation in mature animals. When HCW was included as a co-predictor, model performance improved substantially. The lack of association with collagen content is consistent with tenderness in mature animals being more influenced by connective tissue maturity (e.g., thermally stable collagen crosslinks and/or collagen solubility) than by collagen quantity. Overall, DEXA-derived BMD combined with HCW may complement conventional carcass measures as an objective maturity descriptor and provide moderate prediction of age, collagen solubility and aged tenderness (SF14) in cull cows.

Effect of breed and post-mortem aging time on beef eating quality assessed by untrained Brazilian consumers

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The Brazil Beef Quality (BBQ) program, adapted from the Meat Standards Australia (MSA) grading scheme, has emerged as a promising national framework to predict beef eating quality. The objective of this study was to evaluate the capacity of the BBQ grading framework to predict beef eating quality across major Brazilian regions by assessing consumer sensory responses to Nellore and F1 (Angus × Nellore) beef aged for different post-mortem periods, and to study genotype, aging, and regional effects on consumer perception of palatability. This study provides the first large-scale sensory assessment of beef eating quality in seven states representing the Southeast, Midwest, and South of Brazil. A total of 672 untrained consumers (96 per state) from São Paulo, Goiás, Minas Gerais, Paraná, Rio de Janeiro, Mato Grosso do Sul, and Rio Grande do Sul evaluated meat sensory traits (tenderness, flavor, juiciness, and overall liking) from 12 Nellore and 12 F1 (Angus × Nellore) cattle aged for 5 and 15 days, allowing the identification of patterns of preference and regional differences in consumer perception. Data were processed using the Clipped method to reduce outliers. A Generalized Linear Mixed Model (GLMM) assuming a Gamma distribution was used to analyze fixed effects: the sample tasting sequence, breed (F1 Angus × Nellore, Nellore), aging time (5 and 15 days), consumer region (7 levels), and the interaction between breed and aging time. Consumer and animal were included as random effects to account for within-individual dependence and biological variability. The mixed-model analysis revealed that the interaction between breed and aging time was highly significant for all sensory attributes ($P<0.001$). Increasing aging time from 5 to 15 days induced a significant improvement in scores for both genotypes ($P<0.001$). While F1 (Angus × Nellore) beef consistently received higher scores, the gain observed for Nellore beef aged between 5 and 15 days was substantial. Notably, extended aging time effectively reduced the gap between Bos indicus and crossbred meat, with 15-day aged Nellore achieving sensory scores comparable to 5-day aged F1 (Angus × Nellore) beef. Regarding regional effects, consumer regions did not significantly influence tenderness perception ($P=0.062$). However, a significant regional effect was observed for flavor, juiciness, and overall liking ($P<0.05$), with consumers in Paraná generally assigning higher scores compared to those in Rio Grande do Sul and Mato Grosso do Sul, with differences ranging from 4 to 12 points (on a 0-100 scale). This study demonstrated that Brazilian consumers were able to perceive differences in eating quality between beef from Nellore and F1 (Angus × Nellore) cattle. Crossbred beef consistently received higher scores for all attributes, confirming the superior palatability typically associated with taurine genetics. In addition, post-mortem aging significantly improved the sensory attributes of Nellore beef. These results highlight that controlled aging is a practical and efficient strategy to enhance tenderness and increase consumer acceptance of zebu beef. Despite differences in absolute scoring levels across regions, the relative ranking of genotypes remained consistent across the evaluated states. These findings reinforce the feasibility of adopting a consumer-driven beef grading scheme, such as the BBQ system, to predict and guarantee consistent beef eating quality throughout Brazil.

Bovine appeasing substances before and after transport reduce beef stress

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Dark, Firm, Dry (DFD) meat is a quality defect that reduces sustainability due to its shorter shelf life and consumer rejection, causing important losses to the beef industry. It is characterised by an ultimate pH>6.0, caused by depletion of muscle glycogen prior to slaughter, mainly as a result of stressful conditions experienced by the animal. Reduced glycogen availability leads to an abnormal post-mortem acidification process, resulting in a high ultimate pH. Prior to the slaughter, animals are subjected to transport and lairage, two particular stressful events that may increase energy expenditure and, therefore, contribute to low glycogen reserves in the muscle. Pheromones are substances produced by animals transported through the air that provide communication between individuals. Synthetic pheromones are being used to calm animals in stressful conditions, but their effects on meat quality have been little explored. Therefore, the aim of this study was to assess the effect of applying bovine synthetic appeasing substances (BAS), before and after transport to the abattoir, on the final pH of the meat and on blood stress markers. A total of 144 crossbred entire young bulls from two farms were used. Both farms were located at a similar distance from the same abattoir, and the same lorry and driver transported the animals. Using a syringe, 5 mL of Secure Cattle® was applied to the neck of each animal. A 2×2 factorial design was used, resulting in four treatments: with or without BAS applied on the farm before entering the lorry, and/or applied upon arrival at the abattoir before distribution into the pens. Animals on each sampling day could be assigned only to only one treatment due to the volatile nature of BAS. Transport lasted between 30-40 min. Lairage lasted 14 h, with 13-17 animals kept together in the same pen. After stunning with a captive bolt, blood was collected in tubes during exsanguination and centrifuged 15‘ at 3500 rpm. Serum was stored at -18 $_{o}$C. Cortisol, glucose, creatine kinase, lactate, NEFA and oxytocin were analysed. After 24 h, pH was measured using a penetration probe (XS Instruments). A GLM was applied using SPSS 29.0 with treatment as a fixed effect, and Tukey’s test was used to differentiate significant mean values. The average meat pH when BAS was administered only after transport was significantly higher (P<0.001) than when BAS was applied before transport, either alone or in combination with BAS at the entrance to the abattoir (5.97 vs 5.76/5.82, respectively). This high pH value was associated with a 33.9% incidence of DFD meat, whereas DFD meat was observed only in 13.8% of animals from the control group without BAS, 7.41% in the group receiving BAS only before transport, and 3.70% in the group receiving BAS in both stages. This suggests that applying BAS only before lairage could have a negative effect, possibly due to a reduced ability of animals to adapt to stressors during this period. In the group with the highest DFD incidence, creatine kinase levels were significantly higher (P=0.007) than in the group receiving BAS before transport (608 vs 426 U/L, respectively). Higher creatine kinase levels were associated with lower lactate concentrations, whereas cortisol, which can be considered an indicator of acute stress, glucose and oxytocin were significantly higher in animals from the control group without BAS. NEFA concentrations did not different significantly between groups. Under short-distance transport conditions, BAS may have a positive effect in reducing the incidence of DFD meat when applied on the farm, but may have a negative effect if applied only before lairage. BAS may also improve animal welfare, as indicated by lower blood glucose and cortisol levels.

Authentication of dry and wet aging in Angus beef steaks by means of near-infrared spectroscopy

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In recent years, the Aberdeen Angus breed has been widely adopted due to its ability to improve beef quality, particularly through its high intramuscular fat deposition. Beyond genetics, technological processes such as post-mortem aging play a key role in defining final meat quality. Beef aging is mainly determined by the type of process—dry or wet aging—and its duration. Dry aging is carried out under controlled aerobic conditions of temperature, relative humidity, and airflow, whereas wet aging is performed under vacuum packaging and refrigerated conditions. Despite the higher costs and yield losses associated with dry aging, the aging process is not required to be declared on product labelling. This lack of regulatory protection increases the risk of mislabelling, especially when beef is commercialised as vacuum-packaged steaks, highlighting the need for reliable authentication tools. Therefore, the objective of this study was to evaluate the potential of near-infrared spectroscopy (NIRS), using a portable MicroNIR™ 1700 OnSite-W device, to discriminate between dry- and wet-aged vacuum-packaged Angus beef steaks (picanha, Biceps femoris muscle). A total of 16 picanha cuts (Biceps femoris; 2.52 ± 0.31 kg) from Aberdeen Angus × Charolais heifers slaughtered at 450–500 kg live weight (<12 months) were used. Half of the cuts were subjected to carcass dry aging for 7 days under controlled conditions (2 °C, 80% relative humidity, and 0.35 m/s airflow), then sliced into 2.5 cm-thick steaks and vacuum-packaged. The remaining cuts were directly sliced, vacuum-packaged, and stored under refrigerated conditions for 7 days (wet-aged). In total, 48 vacuum-packaged steak samples were analysed (24 dry-aged and 24 wet-aged). Near-infrared spectra were acquired non-destructively through the packaging using a handheld MicroNIR™ 1700 OnSite-W spectrometer (908–1676 nm). Measurements were performed in direct contact with the packaging film using a zigzag scanning pattern to capture sample variability. Discrimination models between dry- and wet-aged beef steaks were developed using partial least squares discriminant analysis (PLS-DA) with leave-one-out cross-validation. The PLS-DA model showed good discrimination ability between dry- and wet-aged beef steaks, achieving a coefficient of determination in cross-validation (1-VR) of 0.91. Model performance was further supported by the two-dimensional score plot of the first two PLS-DA components, which together explained 99% of the total spectral variability. The score distribution revealed a clear clustering of samples according to aging type, with limited overlap likely attributable to similarities between both aging methods. Overall, these results indicate that portable NIR spectroscopy provides a reliable approach for authenticating the aging process of beef. This approach has strong potential for implementation along the value chain and at the retail level as a rapid, non-destructive tool for the authentication of premium beef steaks.

Evaluation of texture in dry- and wet-aged beef at different pH under various packaging: a preliminary assessment

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Abnormally high-pH meat is associated with an increased risk of developing dark, firm and dry (DFD) meat, which negatively affects quality and consumer acceptance. Tailoring post-mortem ageing strategies according to meat pH at 24 h to identify the most appropriate packaging system could improve meat quality as assessed by instrumental texture. Such optimization is expected to enhance consumer-perceived quality and increase economic returns for producers while reducing losses along the supply chain. This study evaluated instrumental texture in meat with different pH values after dry- or wet-ageing and under different display times. Thirteen carcasses were selected at an abattoir: 8 with normal pH (<5.8) and 5 with high pH (≥6.0). From each carcass, the Longissimus dorsi muscle was excised from both sides. In the normal-pH group, left-side loins were aged as bone-in cuts (dry-aged) for 7 days at 3 $_{o}$C, while right-side loins were deboned at 24 h post-mortem and vacuum-aged (wet-aged) for 7 days at 3 $_{o}$C. The same procedure was applied to the high-pH carcasses. After ageing, the muscles were sliced and packaged using three systems: oxygen-permeable film (FILM), vacuum-skin (SKIN), and modified atmosphere packaging (MAP; 80% O2 and 20% CO2). Samples were displayed in a refrigerated cabinet (3 $_{o}$C ± 1 $_{o}$C) under 12 h of daily illumination for 2, 5, 8 and 12 days, with the day of sampling considered as day 0 of display, and then vacuum-packaged and stored at -18 $_{o}$C. After thawing, 1-cm2 parallelepipeds were analyzed by compression using an INSTRON-4301. Meat cooked in a water bath until reaching 70 $_{o}$C internal temperature was used to obtain shear force with a Warner-Bratzler device on the same instrument. Water losses (WL) were calculated as the difference in weight before and after cooking. Data were analyzed using a GLM (SPSS 29.0) with treatment as a fixed effect and Tukey's test for mean differences. WL were lower in high-pH meat (20.6% vs 23.9%; P<0.001), regardless of ageing, display time or packaging (except SKIN), reflecting greater water-holding capacity, as these conditions allow muscle proteins to retain more water. Wet-aged samples in MAP showed lower WL (20.4%) than dry-aged (24.2%; P<0.005). FILM also reduced WL (P<0.001) at both pH levels by acting as a physical barrier retaining moisture and preventing dehydration. Although dry-ageing is known to improve sensory properties, no differences were observed between dry- and wet-aged meat in shear force or compression at 20% and 80% strain. In contrast, high-pH meat showed significantly lower shear force, regardless of other treatments, suggesting greater tenderness, consistent with our previous findings that such meat has a higher water-holding capacity. Display time reduced meat hardness by over 20% between days 2 and 12 (5.05 vs. 4.0; P<0.05). MAP-packaged meat had higher shear force than FILM (P<0.05), likely due to oxygen-induced protein oxidation increasing cross-linking. Compression at 20% showed more pronounced differences than at 80%, particularly in normal-pH meat, MAP and day 0 of display, as 20% compression reflects muscle fibers resistance and initial elasticity, sensitive to early biochemical changes induced by MAP and pH. In contrast, 80% compression reflects connective tissue resistance and overall structural integrity, which remain largely unaffected, masking subtle differences. Meat pH was the main factor affecting these parameters. Further studies are underway to confirm and extend these findings and guide the meat industry in optimizing processing while enhancing consumer satisfaction.

Incorporating dry-aged beef crust into burger patties: evolution of quality traits over a 7-day retail display

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Dry-aged (DA) beef crust is typically removed, yet its incorporation into patties could valorise trimming losses while modifying product quality during retail display. The present study investigated the effect of incorporating DA crust into 35-day DA beef patties by evaluating physicochemical quality and proximate composition over 7-day retail display storage. Two 35-day DA rib roasts (Austria, male; Poland, female) were trimmed, deboned, ground, and formed into patties weighing 50 g each (n=60; 10 patties/treatment/time point). The 2 treatments were DAN (DA meat without crust) and DAC (60% DA meat with 40% crust). For the 7-day simulated retail display, patties were wrapped in food-grade plastic film and stored at 4.0±1°C and 47±1% relative humidity, under cold LED illumination (natural white 4000 K, 490 lm, CRI>80), for assessment at days 0, 3, and 7. Measurements included pH, colour, drip loss, cooking loss, and proximate composition. Data were analysed by 2-way ANOVA with Treatment, Day, and their interaction (Treatment×Day) as fixed effects. Across retail display, pH increased over time (PD<0.001) and remained higher in DAC than DAN (PT<0.001; day 0: 5.90 vs 5.76; day 7: 6.23 vs 5.91), with a significant Treatment×Day interaction indicating a stronger increase in DAC by day 7 (PTxD<0.001). Lightness (L*) displayed a Treatment×Day (PTxD<0.05): DAN slightly darkened across display (PD<0.05), whereas DAC became lighter by day 7 (42.2 vs 39.8; PT<0.01). Redness (a*) and yellowness (b*) declined from day 0 to days 3-7 (PD<0.05); at day 0, DAC showed lower a* and b* than DAN (a*: 13.4 vs 15.1; b*: 12.9 vs 14.4, PD<0.05), suggesting that crust inclusion altered early colour development. Drip loss increased from days 0-3 to days 3-7 in both treatments (PD<0.001), while the treatment difference was small and only evident during days 0-3 (0.87 vs 0.94%; PT<0.05). Cooking loss declined progressively with display time (PD<0.001) but was consistently lower in DAC (PT<0.001; day 0: 20.7 vs 25.2%; day 7: 13.4 vs 19.9%), and the Treatment×Day interaction (PTxD<0.001) suggests crust inclusion increasingly improved cooking yield as display progressed. Crust inclusion shifted composition at each time point: water was lower in DAC than DAN (D0: 62.7 vs 66.1%; D3: 62.3 vs 66.0%; D7: 62.2 vs 64.6%; PT<0.001), while DAN showed a modest decline over the display period (PD<0.001), with a significant Treatment×Day (PTxD<0.001). Protein decreased over the display period in both treatments (PT<0.001) and was consistently higher in DAC (PT<0.001; D0: 22.6 vs 21.4%; D7: 21.4 vs 20.5%), without a significant Treatment×Day (PTxD>0.05). Fat was higher in DAC across storage (PT<0.001; D0: 7.81 vs 3.87%; D7: 7.62 vs 3.82%); a small change over time was detected only in DAN (PD<0.05), yielding a modest Treatment×Day (PTxD<0.05). Collectively, incorporating 40% DA crust increased fat content and improved cooking yield, but it also shifted colour dynamics during retail display (lower initial a* and b* and higher L* at day 7), indicating a potential trade-off between processability and appearance. To fully characterise shelf-life and consumer acceptability, future studies should evaluate microbiological safety, lipid oxidation, and sensory attributes in crust-incorporated DA patties.

Delivering consistent beef eating quality in Poland: consumer sensory testing supporting the QMP scheme

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In Europe, beef is predominantly traded and valued based on carcass yield and conformation rather than predicted eating quality at the cut level. As a result, consumer eating experiences can be highly variable across muscles and products. Predicting beef eating quality from consumer perception is increasingly recognised as an effective approach to improve product consistency and consumer confidence in beef products and brands. The purpose of this study was to report recent consumer sensory trials conducted in Poland, contributing to the expansion of the national beef eating-quality database and supporting the development of the 3G system aimed at delivering consistent beef meals. The method used was based on internationally harmonised consumer testing protocols recognised by United Nations Economic Commission for Europe (UNECE) for beef eating-quality evaluation. Standardised sample preparation, cooking procedures and questionnaires for untrained consumers ensured comparability with datasets generated within the global 3G consumer-based grading framework. A new series of sensory sessions was conducted over the past two years, during which Polish consumers evaluated beef samples from 15 different muscles across the carcass, covering a range of carcass characteristics and ageing periods typical of commercial production. Consumers assessed tenderness, juiciness, flavour liking and overall liking using 100 mm continuous scales. These attributes were combined to generate a composite eating-quality score expressed as the MQ4 meat quality score. The trials expanded the Polish consumer dataset to more than 13,000 consumers evaluating thousands of beef samples, now the largest consumer sensory database for beef eating quality in the European Union. The dataset provides a strong foundation for analysing variation in eating quality between muscles and for strengthening the 3G predictive model, which estimates expected beef eating quality from carcass and processing measurements. By linking carcass characteristics, muscle type and processing conditions with consumer sensory outcomes, the 3G model enables more reliable prediction of beef eating quality and improved product segmentation across the carcass. The expanded Polish dataset therefore plays a key role in refining prediction accuracy and adapting the system to European production conditions. This work also provides a scientific foundation for the Quality Meat Program (QMP), a national beef quality assurance scheme supported by producers, processors and research institutions. QMP establishes standards across the beef supply chain, including livestock production, feed supply, transport and processing, ensuring traceability and consistent quality. Verified production standards strengthen trust among supply-chain actors and help respond to evolving market expectations. Within this framework, a 3G beef eating-quality prediction system based on consumer research is intended to support both the QMP scheme and the broader transition of the sector toward sustainable production. Together, these elements will underpin the development of the nationwide “Polish Beef” brand, based exclusively on beef originating from certified sustainable production systems meeting requirements for animal welfare, greenhouse gas reduction and full traceability. The official launch of the “Polish Beef” brand is planned for 2027.

Sensory quality and fatty acid composition of beef from Holstein–Friesian bulls finished with different protein sources

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Sensory quality and fatty acid composition of beef from dairy bulls can be modified through dietary strategies applied during the finishing period, particularly by altering protein sources that affect ruminal lipid metabolism. In production systems based on dairy breeds, identifying alternative protein sources that improve meat quality without increasing fat deposition is of particular interest. We hypothesised that replacing rapeseed meal with legume seeds differing in condensed tannin content would modify sensory quality and fatty acid composition of beef without increasing intramuscular fat deposition. The objective of this study was to determine the effects of replacing rapeseed meal (RM) with faba bean grain containing condensed tannins (Vicia faba L.; VF) or with tannin-free white lupin seeds (Lupinus albus L.; LP) in finishing diets for Holstein–Friesian bulls on sensory quality, chemical composition and fatty acid profile of intramuscular fat. Thirty Holstein–Friesian bulls (initial body weight approximately 500 kg; age 17 months) were allocated to three experimental groups (n = 10 each) and fed isoenergetic and isonitrogenous total mixed rations differing only in the protein source of the concentrate for five months. Bulls were slaughtered under commercial conditions, and samples of the longissimus lumborum muscle were collected 96 h post-mortem. After 14 days of wet-ageing, chemical composition, sensory attributes (colour, aroma, juiciness, tenderness and flavour) and the fatty acid profile of intramuscular fat were determined. Data were analysed using one-way ANOVA with dietary treatment as the fixed effect. Replacing rapeseed meal with legume seeds did not affect intramuscular fat content (2.94–3.31%) or collagen content (2.06–2.22 g/100 g; $P > 0.05$). Dietary protein source significantly influenced sensory traits: beef from bulls fed VF diets was rated as more juicy ($P < 0.01$) and more flavourful ($P < 0.05$) than beef from RM-fed bulls, whereas meat from the LP group was characterised by higher tenderness scores than that from the VF group ($P < 0.05$). Dietary protein source also affected the fatty acid composition of intramuscular fat. Inclusion of faba bean grain reduced the proportion of trans-vaccenic acid compared with rapeseed meal ($P < 0.05$), whereas lupin-based diets induced moderate but distinct changes in fatty acid profile. In conclusion, replacing rapeseed meal with legume seeds in finishing diets for Holstein–Friesian bulls modifies beef quality primarily through qualitative changes in lipid metabolism and sensory attributes rather than through increased intramuscular fat deposition. Condensed tannins present in faba bean may play a role in shaping ruminal biohydrogenation and subsequent eating quality of beef.

Predicting beef eating quality: Evaluation by INRAE of an innovative approach to align with consumer and stakeholder expectations

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INRAE has developed a standardised method for evaluating the societal and economic impacts of its research. This method, known as ASIRPA, provides greater insight into how research results are translated into innovation. The objective of this study was to use the ASIRPA method to assess the 3G grading scheme (standing for Global Guaranteed Grading), a system designed to evaluate and predict beef eating quality. First, some historical points were reviewed. The decline in beef consumption is driven by societal concerns, but also by its high price and by the high and inconsistent variability of its eating quality, as shown in consumer surveys. Eating quality depends on many pre-slaughter factors (e.g. sex and age of the animal). It is also influenced by slaughter conditions, carcass characteristics (pH, marbling, etc.), meat cutting and processing as well as meat ageing and cooking. Grading systems take little account of these factors, except the Meat Standards Australia (MSA) system developed in Australia. The system has created added value across the supply chain and ensured a better alignment between price and eating quality from the consumer's perspective. According to the 2024–2025 report, the MSA system generated 409 million Australian dollars (€245 million) in additional value for the more than 13,000 Australian farmers. Then, the ASIRPA method described how the principles of the MSA system have been investigated in Europe, taking into account the diversity of livestock systems and consumer expectations in several European countries (Poland, Ireland, France and the United Kingdom), where companies are exploring its use to enhance product quality. This research was carried out as part of over five research projects coordinated by INRAE or its partners, including the national EcoRegMeat3G project and the European INTAQT project, both led by INRAE. The work of five PhD students, all supervised by researchers from INRAE also contributed to this work. This research led to the development of the 3G system managed by the International Meat Research 3G Foundation established in Poland in 2017 and operating with the support of INRAE. It also contributed to the launch of a new premium beef brand (OR ROUGE) by the Beauvallet beef company in the French Limousin region. Beauvallet is engaging with the 3G system in the context of developing its new brand for high-quality Limousin beef, with improved farmer remuneration. In addition, the 3G system has been successfully evaluated in South Africa, South Korea, Japan, the United States and New Zealand. Worldwide, economic analyses indicate that the Meat 3G system generates added value of between €0.10 and €0.55 per kilogram of boneless beef for farmers. By enabling more accurate grading of meat, the system reduces economic losses while strengthening the competitiveness of the sector. The development and wider deployment of the system are currently overseen by the International Meat Research 3G Foundation in collaboration with the French company Birkenwood Europe, founded in 2021. Building on this work, the United Nations Economic Commission for Europe (UNECE) has issued new recommendations on beef eating quality that draw on the principles underpinning the 3G approach.

Participatory and iterative approach to design collective strategies of crop-livestock integration

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Climate change, ecological pressures, economic uncertainties and social expectations are increasing the vulnerability of livestock farming systems. Crop-livestock complementarities are seen as a solution to these challenges, but their consolidation and territorial scaling remain challenging. This paper describes and discusses an iterative participatory design framework developed to support such collective strategies and implemented in South-Western France. The study area is characterized by specialized viticulture and long-term pastoral disuse, leading to shrub encroachment, reduced biodiversity and weak local food provisioning. In response, several local initiatives have emerged to reintroduce livestock into vineyard landscapes and to rebuild crop-livestock complementarities. The participatory process combined a territorial agroecology perspective with an innovation ecosystem approach, aiming to connect research, advisory services, local authorities and practitioners around shared problems and concrete opportunities. First, field diagnosis and qualitative inquiry were used to document existing crop-livestock initiatives, identify success and failure conditions, and understand the diversity of actor perceptions and organizational arrangements. Second, biophysical assessments were conducted to estimate forage potential, grazing capacity and seasonal resource availability across agricultural, semi-natural and abandoned land uses. Third, design and exploration tools were mobilized, including serious game, consultation meetings and scenario-building workshops, to support collective reflection on technical, organizational and territorial options. Finally, simulation and evaluation were used to assess scaling-up potential, nutrient cycling, economic performance and the capacity of the landscape to sustain livestock reintroduction over time. Results show that crop-livestock integration in Mediterranean vineyard regions cannot rely on a single technical dimension. Instead, long-term viability depends on combining multiple levers: diversified forage resources, mobility of herds, access arrangements, stakeholder intermediation, public support, and local coordination. It also showed that practical solutions must be tailored to different scales, from vineyard estates to municipalities and larger territorial units. This study explore how an adaptive, participatory and multi-level design framework can support co-innovation in complex crop-livestock systems, while producing both context-specific solutions and transferable lessons. By combining qualitative and quantitative approaches, and by involving a complete innovation ecosystem, the project created conditions for dialogue, learning and collective action. This approach helps bridge transition studies, agronomy and territorial design, and offers pathways to strengthen resilient, multifunctional and low-input agricultural systems. Nevertheless, a reflective analysis of the co-design process implemented appears necessary in order to adapt it as effectively as possible to other situations. Extending this approach to other livestock farming contexts also seems relevant for examining the contribution of livestock farming to sustainable regional development, particularly in light of the challenges posed by planetary boundaries.

Livestock system transformation as a societal task: governance lessons from a stalled multi-stakeholder strategy in Germany

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Current livestock production systems are increasingly scrutinised due to their environmental and climate impacts and concerns about animal welfare. While there is broad consensus that livestock systems need to become more sustainable and animal-friendly, there is far less agreement on how feasible transformation pathways can be implemented in practice. This paper analyses why a broadly supported transformation strategy for the German livestock sector failed to translate into political implementation and derives governance-related lessons for future transition processes. The analysis is based on the experience of the German Commission on the Future of Agriculture (Zukunftskommission Landwirtschaft, ZKL), a multi-stakeholder initiative established by the federal government that brought together representatives from agriculture, the food industry, environmental and animal welfare organisations, science, and civil society. The commission developed a consensual strategy for transforming livestock production towards higher sustainability and animal welfare standards. A key outcome was the recognition that achieving these goals would require long-term financial support to compensate farmers for investments and increased production costs exceeding world market prices. Despite broad acceptance of the commission's recommendations among stakeholder groups, including the farming community, implementation has largely stalled. The analysis identifies several structural reasons: limited political ownership beyond a single ministry, absence of binding long-term financing mechanisms, misalignment between political decision cycles and agricultural investment horizons, insufficient cross-party consensus on how to distribute transformation costs between taxpayers and consumers, and institutional fragmentation across policy domains. The lack of political follow-up after the commission process created a governance vacuum that contributed to considerable investment uncertainty in the livestock sector, resulting in postponed investments, declining livestock numbers, and reduced production capacity. The German case illustrates that livestock transformation is not only a technical or sectoral challenge but primarily a systemic governance problem characterised by conflicting societal goals, including food affordability, economic viability of farms, environmental protection, and animal welfare. Transformation processes therefore require negotiated societal compromises rather than purely technical solutions. Even well-designed multi-stakeholder processes with broad consensus may fail without strong political commitment, institutional continuity, and societal agreement on financing public goods associated with sustainable food production. These findings underline that livestock transitions cannot be achieved by farmers alone but require coordinated action across the entire agri-food system, supported by coherent policy frameworks, long-term financing strategies, and broad societal responsibility. The lessons derived from the German experience may inform transformation processes in other livestock-producing regions facing similar governance challenges.

A Biocultural Diversity Index for Assessing Animal Husbandry Sustainability

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Once defined as biological, cultural, and linguistic diversity which are interrelated within a complex social-ecological adaptive system, biocultural diversity (BCD) describes the co-dependence of cultural and biological diversity within social-ecological systems. The BCD Framework structures social-ecological systems to be made up of materialized, lived, and stewardship components contributing to biocultural diversity. Globalization and simplification of animal husbandry systems are contributing to biocultural homogenization, which refers to the loss of BCD through the homogenization of anthropogenic habits and knowledge and the creation of globally homogenous natural environments. Today, highly integrated and industrialized are increasingly replacing traditional animal husbandry systems in local, national, and global markets. Shifting systems has large implications for biocultural homogenization. For example, meat consumption has changed drastically within the past decades. Global meat production has surged through technological advancements where indigenous and local products are being replaced by global imports or Western production systems, leading to a shift in consumer preferences and further supporting global simplification. Overall, biocultural homogenization degrades sector as well as societal resilience, inclusivity, and the potential for sustainable transformation. In our previous work, we illustrate specific animal husbandry systems in Europe as biocultural diversity storehouses that contribute to biodiversity conservation, climate resilience and cultural values. The work is highly theoretical, yet provides a strong framework for elaborating BCD to understand transformation potential and holistically assess livestock systems' sustainability. Next steps, involve collecting and creating (semi-)quantifiable indicators for a BCD index for monitoring and comparing materialized, lived, and stewardship components of BCD across husbandry systems, regions, and time. A preliminary sustainability index is elaborated with stakeholders to reflect and assess poultry production through the BCD lens. A triangulation perspective of stakeholders covering production (e.g. organic and conventional producers), processing and retail (e.g. representatives from slaughterhouse and retail), and stewardship bodies (e.g. municipal governments, nature conservation groups, breeding associations) as well as researchers from the disciplines of social-ecological systems and poultry science are brought together to collect, rank, and weight indicators across the three components of BCD. First, stakeholders are introduced to the concept of BCD and the BCD Framework as part of a brief guided-question focus group discussion on poultry production sustainability. Next, a world café format commences brainstorming and a discussion on indicators' feasibility. Finally, indicators are then ranked and weighted based on consensus-based decision-making. The resulting index is summarized by the research team as an inter- and transdisciplinary approach for comprehensively understanding animal husbandry system sustainability. Acknowledgements: the Lower Saxony Ministry of Science and Culture and the Volkswagen Foundation funded this research as part of the zukunft.niedersachsen program.

Multicriteria evaluation of mixed sheep-beef production systems

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Multicriteria evaluation is a mean to assess the durability of farming systems. Several methods and evaluation trees have been proposed for a variety of projects and objectives. So far most methods have focussed on specialised systems. Yet improving the synergy between beef and sheep enterprises within and between farms seems to have several assets such as a better economic resilience, a better valorisation of the fodder resources and an improve management of parasitism in sheep. In the project ACCOMPLIR, to assess the overall impact of sheep – beef mixed systems on farm durability, we proposed and tested a multicriteria evaluation tree and methodology. To consider the specificities of mixed systems, the methodology proposed some new indicators such as the Shannon Index and the proportion of the main enterprise in the gross product of the farm to quality the diversification of the farming system. In addition, since product units commonly used in sheep and beef production are not directly comparable (resp. / kg. of Carcass equivalent and /kg of live weight), we choose to evaluate the environmental indicators in relation to surface areas. Finally, with references only on specialised systems, each farm had 2 scores for each indicator. To combine those to scores in a single score, we prorated the results to the e were able to compare the mixed farms results to specialised sheep and beef farms, and to have a single score for each indicator, we prorated the result to the number of livestock unit between the two enterprises. Two ponderations between indicators and criteria have been tested, an Equi ponderation and a ponderation with a panel of 21 experts, and technicians. There was a significative difference only for the viability criteria between the 2 ponderation methods. We choose to keep the ponderation proposed by experts and stakeholders to favour a better understanding of the evaluation method. For aggregation between indicators, we compared two options: calculating the average of indicator and criterion scores on the one hand and limiting compensation between indicators using CONTRA software on the other. The actors involved in the project favour the limitation of compensation. This methodology was tested on 15 sheep-beef French mixed farms and highlighted that those farms were on average more resilient to risks and had a better economic viability yet were less transmissible than specialised systems on the economic pilar and were comparable to suckler farms without fattening systems on the environmental pilar. The comparison with specialised systems was not available on the social pilar due to the creation of a questionnaire for the needs of the project. In the future, the evaluation tree needs some adaptation for the social pilar to better consider animal welfare. In addition, there is a need to expand the method to include more farm data to establish references for mixed farms so that these systems can be compared with each other and the comparison with specialized systems can be strengthened.

Network Focus Animal Welfare – how to transfer practice-orientated knowledge into practice

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Germany lacked a structure that bundled and processed knowledge about animal welfare and made it accessible to livestock farmers. The solution was funded by the Federal Ministry of Food and Agriculture since 2020. The nationwide "Fokus Tierwohl" network aims to improve the transfer of knowledge on animal welfare into the agricultural practice, taking sustainability and environmental protection into account. For the first time, all federal states participated in this network, so that the knowledge could be coordinated nationwide. A focus was on promoting networking between experts and farmers. Specialist knowledge is bundled and made freely available, an exchange of experience between practitioners, scientists, consultants and other groups is organized, thus enabling and promoting networking within the industry. The target groups are employees on farms, in the upstream and downstream sectors, advisors, veterinarians and the interested public. Members of the network have access to a nationwide common calendar and a document sharing system. Coordinated monthly meetings, standard operation procedures and corporate identities ensure a joint appearance. A nationwide expert advisory board has been established in 2020, they selected around 900 animal welfare topics, including sustainability and environmental protection. Working groups – consisting of scientists, farmers, consultants and representatives of the authorities – started selecting, bundling and summarizing the newest information to make it available for the target groups. Animal welfare multipliers organize various events to disseminate the knowledge in each federal state. These vary from (farm) workshops to school visits, from seminars and fairs to farmers markets. Due to the different target groups, different ways of knowledge transfer have been established. Beside scientific articles, checklists, guidelines, training documents, virtual farmwalks, videos and podcasts are available. Given that farm staff originate from different nationalities, translations in English, Polish, Romanian and Ukrainian are available. About 120 demonstration farms complete the network. They are pioneers in the profession with innovative and sustainable animal welfare concepts and present their concepts at trade fairs and events. Practice-orientated knowledge is getting into practice, while the farmers network with each other, visit farms in Germany and abroad. In general, the three following ways of dissemination are used: 1) events of the network partners (so far 3.000 events with more than 130.000 participants); 2) website: www.fokus-tierwohl.de and 3) network of demonstration farms. The website provides links to research results, from funded research and development projects or the model and demonstration projects on animal welfare as well as other stakeholders. A very well-organized database is available and can continue to be used across borders. For the first time in Germany, a nationwide functioning network for knowledge exchange has been established. It can be used as a platform to disseminate results from all projects relating to animal welfare, sustainability and environmental protection and there are no technical obstacles to expanding it to other topics. This network can be used as a 'blueprint' for knowledge hubs in other areas.

How do livestock farming system issues come into play in the design of breeding programs for agroecology? Key points from researchers'narratives of their experiences in a transdisciplinary project

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Several syntheses about genetic selection of livestock for agroecological transition have underlined key challenges for breeding activity and researches in genetics, like relevant breeding goals or role of diversity. Beyond these issues, agroecology involves to be context-sensitive, to do with the ecosystem processes rather than to control the environment, to take into account biological interactions etc. Consequently, as mentioned in the literature, this involves to reconsider genetic selection in a systemic perspective. However, little is known about how those principles are translated into actions in the fields, when co-designing breeding programs in an agroecological perspective. Particularly, how do livestock farming system issues come into play in the design of breeding programs for agroecology? To contribute to answer this question, we rely upon the analysis of narratives of research experiences produced by an interdisciplinary group of researchers gathered, in the frame of a project named 'Cobreeding', in a task to design and test various combinations of genetic resources in farming systems coupling plants and poultry. The narratives were produced through semi structured interviews, transcribed and used as material for a thematic analysis. It was followed by a workshop to explore the convergences and specificities identified in the experiences reported by researchers of this task. We highlighted several modalities through which genetic dimensions are invoked in livestock farming system design, and, reciprocally, how livestock farming system dimensions are invoked in genetic research. We emphasized several livestock farming systems dimensions, in line with agroecological principles, which are at play at the farm scale (e.g. which genetic resources to promote an autonomous feeding system) or challenges at higher levels of organization. We finally showed that the temporality of the co-definition of research questions and relevant experiments is not to neglect, to build convergence between (i) the challenges identified by scientists, relying on generic knowledge as well as their past researches' experiences, and (ii) challenges expressed by farmers in the specific context of the case study. For instance, if farmers were interested in the role of poultry to predate insects in orchard, they did not express wishes to work on the associated selection criterion. On the other hand, interest for selection criterion to face predation of poultry by birds of prey seemed more pronounced. We then discuss the interests and difficulties associated with such interdisciplinary and transdisciplinary set ups, and how they potentially put at play new research questions and practices (systems diversity, scales and levels of organization, targets and conceptions underlying the breeding goals, identification of relevant breeding criteria etc.). Finally, we emphasize the interest to extend this work to other tasks of the Cobreeding project, like the one on the re-conception of cattle breeding programs to meet the challenges of climate change.

Co-designing climate adaptation strategies in PDO/PGI livestock territories: a typology of participatory and multi-level approaches from the ADAoPT project

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The ADAoPT project supports Geographical Indications (GIs) livestock value chains in the design of climate adaptation strategies. Thanks to 6 case studies, the project sought to explore how participatory and multi-level dynamics can structure strategic reflection in GI livestock systems. The 6 territories differ in production systems, organisational structures, exposure to climatic risks and prior experience on climate issues. To initiate the process, each GI first established a common reference framework (delineation of the perimeter, governance arrangements, time horizon, available resources, existing knowledge and strategic objectives). Consequently, the themes addressed and the composition of working groups varied. Rather than applying a standardised methodology, each GI developed a pathway reflecting its resources, constraints and ambitions. Cross-analysis of the six experiences led to a two-dimensional framework for characterising adaptation methodologies. The first dimension, Spatial–Functional, concerns the scale and composition of mobilised groups. A spatial approach engages a broad diversity of actors representative of the territory (farmers, processors, technical advisors, local authorities). A functional approach relies on a smaller, task-oriented group focusing on a specific mission or expertise. The second dimension, Thematic–Systemic, relates to the breadth of issues addressed. Thematic approaches focus on a single issue (e.g. water, forage, energy), whereas systemic approaches integrate interconnected challenges such as natural resources, biodiversity and labour. Combining these axes results in four configurations:(1) Thematic–Functional: a restricted group explores a targeted issue to produce operational tools and build focused expertise.(2) Systemic–Functional: a small governance-linked group develops a comprehensive strategic vision for the GI.(3) Systemic–Spatial: territory-wide mobilisation supports shared diagnosis, scenario-building and cross-actor dialogue.(4) Thematic–Spatial: a specific issue is addressed through broad participation, fostering experimentation and connections between value chain segments. In practice, territories rarely remained within a single configuration. They navigated between them according to emerging needs, available capacities and decision timelines. Alternation between systemic and thematic phases proved particularly important: global reflection helps prioritise and align ambitions, while thematic focus translates vision into actionable plans. This iterative movement supports collective learning and progressive alignment. A central challenge lies in articulating diverse initiatives into a coherent roadmap. Actions often address specific facets of climate change, yet their cumulative contribution depends on explicit coordination and strategic integration. Governance appears as the keystone of this articulation across scales and groups. Combining broad participation with specialised working groups enriches deliberation but may generate tensions regarding representation and decision-making. Clarifying roles, consultation procedures and arbitration mechanisms is therefore critical to ensure legitimacy. The ADAoPT experience shows that effective adaptation in GI livestock systems does not rely on a single participatory model. It emerges from context-sensitive combinations of spatial and functional groups, and from deliberate alternation between systemic and thematic approaches, embedded in transparent governance arrangements.

Lessons learned from living labs on Swiss and Italian beef production in the Project "INnovative Tools for Assessment and Authentication of chicken meat, beef and dairy products' QualiTies"

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The INTAQT project (EU Horizon 2020) studies the relationship between animal husbandry systems and the quality of animal products. Within the project two farmer field-groups (living labs) were established, one in Switzerland and one in northern Italy involving 5 farms each, on the topic of sustainable beef production. In Switzerland, crossbred calves from the dairy sector are moved to fattening farms, where they are extensively fattened until the age of 24 month, using at least 75% of grass in the diet. In Italy, conventional beef farms are based on intensive fattening of young bulls, fed with a total mixed ration based on corn silage and concentrates. The aim of the living labs was to implement on-farm changes in the production processes (e.g. feeding regimes, outdoor access, herd management) expected to improve intrinsic quality traits of the products and/or sustainability traits of the farms. From 2023 to 2025 farmers within each living lab met 2-3 times on each other's farms, allowing discussions about their experiences, successes and drawbacks; the present contribution summarized lessons learned from these two living labs. In Switzerland, during the first year of the living lab, participants identified as main obstacle to the current production system the large genetic variety of the animals, which limits their ability to achieve a homogenous carcase quality. As a result, participating farms requested a broader research project on this topic, which lead to increased cooperation and data-sharing between the leading researchers, fatteners and the value chain. The second meeting, highlighted challenges regarding feed supply in dry summers and limited growth rates during the alpine estivation of animals. Jointly, farmers developed strategies to overcome drought and feeding challenges, such as the pre-emptive production of maize silage, the switch to breeds, such as Angus, which are better adapted to extensive feeding and increased farm collaborations. The third living lab meeting identified the supply of cross-bred organic calves as important barrier to the growth of the sector. This eventually led to an active involvement in the restructuring of the fattening sector as well as the establishment of a second research project including not only the beef production value chain but also stakeholders from the dairy industry. In Italy, the aim of the living lab was to co-develop protocols and guidelines for the implementation of a certification of "Sustainable beef"; meetings and on farm visits were organised, and the outputs of the process were the definition of specific advisory services and the establishment of a demo farm to favour the implementation of good practices (such us low impact diets, use of beef on dairy, reduction of antibiotic). The experience of both living labs highlights the importance of allowing a farmer lead research approach by establishing trust, ensuring active involvement of researchers and allowing flexibility in the definition of the living lab's framework. This resulted in a fertile research ecosystem in which farmers perceive to be actors rather than subjects of the process.

SPICER² : a multi-partners network to promote the improvment of Cultures-Livestock Interactions in the French agriculture

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The SPACER² Network follows on from the SPyCE and SPICEE Networks. It aims to strengthen understanding and promote Crops – Livestock Interactions (CLI). French agriculture is increasingly subject to climatic and geopolitical uncertainties, which call into question the functioning of systems that rely heavily on the consumption of non-renewable or low-renewable resources (minerals, soil) and fossil fuels. An agroecological transition towards more sober, robust and resilient systems can be facilitated by increasing interactions between crops and livestock, increasing the circularity both at the farm level and among collectives across territories.The overall objective is to promote agricultural systems that are more autonomous and resilient to climatic and economic uncertainties by involving a wider range of actors, including those involved in agricultural research, training and development, as well as new actors, whether agricultural or not, who are involved in agriculture in the regions. The programme is structured around four areas: one steering area and three thematic areas. The latter focus on (1) capitalising on and disseminating knowledge, (2) documenting, monitoring and designing new forms of crop-livestock organisations, and (3) evaluating their contributions to the performance of farms, sectors and regions in a fluctuating economic and climatic environment.

Enhancing interoperability through transparent multidirectional data architecture for individual animal data valorization in the pig value chain

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Digital transformation holds great promise for sustainable, resilient pig production systems. Yet, fragmented data use hampers progress. Current systems suffer from isolated solutions, unclear data ownership, inconsistent recording, missing interfaces and limited individual animal-level linkage, preventing effective value creation. The German pig sector faces intense competition from international imports while needing to meet rising demands for animal welfare, meat quality, transparency and resource efficiency. This project addresses the interoperability along the pig production value chain by developing a transparent, multidirectional data architecture connecting all value chain stages via individual animal identification. The three-year project will run until the end of 2027. Core is a large-scale UHF-RFID implementation across the chain, enabling automated data capture from genetics through slaughter. In two different farms, existing infrastructure (animal feeders, handling areas) is supplemented by UHF-RFID readers and 3D-cameras for individual performance and weight estimation. Piglets receive individual tagging (UHF-RFID) for system comparison across 4-6 fattening cycles. Automated antennas at loading/unloading points ensure seamless tracking through transport to slaughterhouse intake. Within the slaughterhouse multiple antennas and sensors for a gapless recording of the individual animal are also installed. Additional qualitative and quantitative information about the carcasses is also recorded in the slaughter house along the processing chain. Individual animal identification is the connecting link that makes all other information along the chain compatible with each other. In addition, the possibility of integrating new automated quality measurements is being examined. These measurements allow for more detailed evaluations thanks to traceability back to individual animals. This can be used to determine new information and create added value e.g. in the context of breeding and feeding. A central digital platform serves as an interoperability hub, harmonizing farm management, logistics and slaughter data while preserving data sovereignty through role-based access. Individual data is not stored on a central platform but rather accessed, retrieved, and distributed in a targeted manner as in a transshipment station. Therefore animal-level data (genetics, growth curves, treatments, transport quality, meat quality traits like intramuscular fat content as eating quality proxy) becomes linkable for holistic analyses. Expected outcomes are for example enhanced planning for the farmer and the slaughter house, resource efficiency (logistics matching), animal health/welfare monitoring and quality-based payment. Interoperable data flows via digital delivery notes enable data monetization, supporting “less but better meat”, breeding for sensory quality, and differentiation from commodity products. This strengthens economic resilience and transparent value chains. The GlasSchwein project is funded by the German Federal Office for Agriculture and Food (Project ID 28DI107B23).

Supporting Farmers in Developing Systems with Higher Human-Edible Protein Output: Balancing Animal and Crop Production at Farm Level

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Ruminants are often criticized as inefficient protein producers, despite their ability to utilize grassland unsuitable for crop production. The objectives of the Interreg Proscor project are: (1) to select indicators to measure protein production efficiency and land use on cattle farms; (2) to involve farmers in redesigning systems that maximize protein production for human consumption; and (3) to disseminate a simple tool and method for estimating farm efficiency and identifying improvement opportunities. Five indicators were selected: edible protein feed conversion efficiency (epFCE), land use ratio (LUR), protein autonomy (PA), global protein yield (GPY) and net protein yield of the production system (nPYPS). Two groups of dairy cattle farmers (9 French and 8 Swiss farms) and two groups of beef cattle farmers (5 French and 7 Swiss farms) were formed. Technical and economic data from 2023 were used to calculate the indicators. Farmers were brought together repeatedly in 2025 and 2026 to contribute to indicator analysis and refinement, and to develop scenarios for redesigning their systems. Sharing reference values for human-edible protein from various crops and animal products allowed farmers to consider new crop rotations adapted to their pedoclimatic context. These rotations incorporate crops with a high potential for human-edible protein (grain legumes, cereals, potatoes), along with catch crops and pastures used to feed animals and produce animal protein. Farmers could thus explore a new crop–herd balance, identifying arable land suitable for crops and areas to remain under pasture. Initial findings indicate that in three-quarters of the scenarios, herd size or milk and meat production was reduced. For the least efficient systems for producing human-edible protein (notably suckler systems), redesigning the system to produce both plant and animal protein simultaneously increases human-edible protein production and consequently reduces feed–food competition. The economic aspects of these changes are being considered to assess system viability. Farmers identified key barriers to implementing these scenarios, including limited time to manage new production activities, investment needs for livestock buildings, availability of equipment for new crops, lack of marketing channels, and climate-related uncertainties affecting legume crops from year to year. These results suggest that the levers for improving protein efficiency on farms will need to be deployed at the regional level.

Understanding drivers and barriers for implementing grassland and feeding measures to reduce ammonia and methane emissions in Dutch dairy farming

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Reducing ammonia (NH_3) and methane (CH_4) emissions from dairy systems requires the implementation of technical mitigation measures at farm level. However, adoption depends not only on technical feasibility but also on farmers' perceptions, motivations and structural constraints. This study aimed to identify opportunities, drivers and barriers influencing the implementation of grassland and feeding strategies to mitigate NH_3 and CH_4 emissions in Dutch dairy farming. The study is part of a nationally funded project (https://www.sia-projecten.nl/project/integrale-aanpak-ammoniak-en-methaan) and consisted of a literature review combined with a baseline assessment (2023). Data were collected through online surveys (106 dairy farmers, 98 animal husbandry students and 51 sector stakeholders) and in-depth interviews with farmers and sector representatives. Survey respondents evaluated their attitude towards various mitigation measures using a five-point Likert scale and reflected on perceived feasibility, risks and incentives. Attitudes towards potentially promising measures differed substantially. Feeding-related measures such as increasing concentrate proportion (mean score 2.35) and the use of nitrate (2.78) or 3-NOP-based additives (2.92) were viewed more critically. In contrast, management-based measures such as optimised mowing strategies (3.49), increased grazing (3.58) and multispecies grasslands (3.60) were perceived more positively. Interviews revealed that many farmers already apply certain mitigation practices unconsciously, indicating hidden opportunities for emission reduction. At the same time, knowledge on emission-reduction mechanisms was often fragmented, and uncertainty about policy goals and effective strategies limited targeted action. The survey revealed key drivers and barriers influencing the implementation of emission-reducing measures in grassland management and livestock feeding. Key drivers included less costs/higher revenues, animal welfare/animal health, the image of the livestock sector, own norms and values, and less labour/better working conditions. Important barriers were perceived animal welfare/animal health risks, financial constraints, regulatory uncertainty, lack of knowledge/education, and time/available labour. Multiple "lock-ins" were identified in the in-depth interviews, where existing infrastructure, investments or established habits restrict the ability to change practices. Across groups, farmers tended to perceive more threats than opportunities in current emission-reduction debates. The findings demonstrate that successful emission mitigation requires alignment with farmers' intrinsic motivations and farm systems rather than focusing solely on technical optimisation. Addressing and unlocking systemic lock-ins and clarifying long-term policy objectives appear crucial to enhance effective implementation of NH_3 and CH_4 mitigation measures in dairy systems.

Integrated assessment of sustainability indicators in pasture-based and intensive dairy sheep systems in Central Italy

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Dairy sheep farming in Mediterranean areas ranges from pasture-based to fully intensive indoor systems. In Central Italy, pasture-based farms are typically semi-extensive, with grazing during favourable seasons and indoor housing from late October to March. Although pasture access is often associated with improved animal welfare, farm sustainability is influenced by management practices, housing conditions and seasonal variability. Therefore, integrated assessments are needed to support sustainable dairy sheep farming. This study aimed to compare pasture-based and intensive dairy sheep systems in terms of animal welfare and environmental, economic and social sustainability. The study was conducted within the EUPAHW (European Partnership on Animal Health and Welfare) framework and focused on case studies analysing trade-offs between sustainability dimensions and animal welfare. The study was carried out in the Abruzzo region (Central Italy) over one year, covering all seasons. At the current stage, five commercial farms were evaluated during winter: two pasture-based (semi-extensive) and three intensive system, all rearing Lacaune sheep. Pasture-based farms included about 120 lactating ewes each, while the intensive farm housed about 150. During winter, animals in pasture-based systems were also kept indoors. Animal welfare assessment was based on health and behavioural indicators. Health indicators included body condition score, lameness prevalence assessed through visual scoring and mobility evaluation, mastitis incidence and somatic cell count, parasitic burden, and heat stress evaluated using the Temperature–Humidity Index (THI). Behavioural indicators focused on activity patterns, social interactions and stress-related behaviours. Environmental sustainability was assessed using life cycle assessment–related parameters, focusing on feed management, manure handling, water consumption and land use. Economic sustainability was evaluated through indicators of production efficiency and resource use, while social sustainability was assessed through structured questionnaires addressing labour organization, workload, occupational health and safety, perceived work-related stress, farmer-animal interaction and job satisfaction. Preliminary winter results showed that welfare and management criticalities were more evident in pasture-based systems during the housing period, with greater variability in housing structures and health management practices compared with intensive systems. These findings highlight the importance of considering seasonal housing conditions and management strategies when assessing the sustainability of pasture-based dairy sheep systems. This work was co-funded by the European Union's Horizon Europe Project 101136346 EUPAHW. Views and opinions expressed are those of the author(s) only and do not necessarily reflect those of the European Union or the European Research Executive Agency.

Evaluation of animal-level strategies for reducing nitrogen and phosphorus losses and their effectiveness in mitigating the environmental burden of pig production

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Dietary nitrogen (N) and Phosphorus (P) are essential nutrients to animals, and the produced fecal and urinary N and P are essential fertilizers for plants. When the agronomic nutrient cycle becomes unbalance in regions with high livestock densities, losses of N and P lead to a range of environmental impacts, like water eutrophication of coastal, freshwater and groundwater ecosystems as well as soil acidification. Efforts have been made by the pig sector to mitigate these impacts but it is necessary to go further. For instance, the pig science community has (1) developed strategies aimed at improving nitrogen (N) and phosphorus (P) use efficiency in pigs to reduce nutrient excretion without compromising animal performance, and (2) evaluated the environmental performance of these strategies through Life Cycle Assessment (LCA) to quantify their effectiveness. This review focuses on fattening pigs, which contribute the most to N and P losses. Reduction of crude protein (CP) content of diets (-1 to -1.5 pt relative to current practice) with balanced supply of digestible amino acids (AA) has proven to be effective in reducing N excretion while maintaining animal performance. Further reducing CP content has recently produced more contrasted results on animal performance and N emissions. For P, the use of phytases is widely recognized to improve P digestibility, what makes possible to reduce P excretion. The strategy of depletion – repletion can further improve P utilization efficiency and lower P excretion without affecting growth. In addition, precision feeding, by providing to each animal each day tailored supplies in digestible amino acids and digestible P, has also proven to be effective in reducing N and P excretion. To establish the effectiveness of these strategies, LCA inventories the resources and emissions along the life cycle of the product, from the inputs for feed ingredients production to the live pig at farm gate, including feed manufacturing, emissions from animals and manure, and all the transports steps. In this field of research, the perimeter usually also includes emissions at field application of manure and the economy of mineral fertilizer permitted by manure. With LCA, the potential of these strategies depends on 1) the set of the environmental impacts considered 2) the relative impacts of the feed ingredients substituted and the ingredients incorporated in the mitigation strategy, 3) the origin and environmental impacts of feed-grade amino acids, 4) the levels of digestible amino acids, digestible P, CP and total P contents in the reference strategy and 5) the practices for field application of manure. To overcome the limits of these strategies, there is further interest in associating them with multi-objective feed formulation and good environmental practices for manure management (e.g. frequent manure removal from buildings). In addition, the amount of manure applied on field is largely determined by the N to P ratio of manure relative to crop requirements. If the ratio is unbalanced, more mineral fertilizer will be applied, and the benefits obtained from mitigation strategies implemented in pig production could thus be negated. Therefore, the effective application of strategies targeting N and/or P losses should be also analyzed in the next future with a broader view at farm and territory levels.

Variation in N and P excretion from sows, weaners and grower-finisher pigs

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Surplus of nitrogen (N) and phosphorus (P) from production of livestock and associated application of manure can cause eutrophication. Estimated amounts of N and P in manure from pigs are used by Danish farmers in a manure accounting system to document surplus or deficit of N or P for the land owned by the farm. If there is a surplus the farm must export manure, while if there is a deficit, the farm is allowed to buy fertilizer or receive manure from another livestock farm. The calculated excretion of N and P per sow per year or per produced pig is based on average values. However, there is variation in efficiency between farms. The aim of the study was to investigate the variation between farms based on farm data for feed intake and expected difference in crude protein (CP) and P content in the feed as well as emission of N from the barn. The ex-storage excretions of N and P used in the Danish manure accounting system are based on mass balances. Input of feed, number of piglets born, weaning weight and weight gain for weaners (6.4 kg to 31 kg) and grower-finisher pigs (31-117 kg) are reported by the farmers. The expected N and P content in feed is based on a survey involving the major feed companies in Denmark. The N and P contents per kg of weight gain are based on literature values. Emissions of N in the barn and storage system are subtracted and inputs of N and P in bedding are added. Data was obtained from 716 sow farms, 422 weaner farms and 1111 grower-finisher farms covering approximately 63%, 39% and 73% of the sow, weaner and grower-finisher farms in Denmark. The farms were divided into five percentile groups of equal size based on feed intake, and within each percentile group average feed intake was calculated. The same productivity was used in each percentile group. Three different CP and P content in feed (low, medium and high) were applied for each feed intake percentile group, based on theoretical recommendations for sows and growing-finisher pigs, while six different levels of CP and P were used for weaners. Three different housing emission values for ammonia were applied. All other parameters were kept constant. That gave in total 45 different combinations of feed intake, feed composition and barn emission for sows and grower-finisher pigs and 90 for weaners. The mean, standard deviation and coefficient of variation (CV) were calculated for ex-animal, ex-storage and intake of N and P. The ex-storage values for sows were on average 19.3 kg N/sow/year and 4.76 kg P/sow/year and the CV was 12.5% and 14.9%, respectively. The ex-storage values per produced weaner were on average 0.37 kg N and 0.091 kg P and the CV was 17.9 and 17.0%, respectively. The ex-storage values per produced grower-finisher pig were on average 2.32 kg N and 0.43 kg P and the CV was 6.7% and 9.3%, respectively. The CV for the ex-animal and ex-storage within N and P were nearly identical. This indicates the main source of variation in manure N and P ex-storage is variation in feed efficiency and CP and P content of the feed. The N-intake was on average 29.6 kg N/sow/year, 1.1 kg N/weaner and 5.3 kg N/grower-finisher pig with a CV on 5.9%, 6.4% and 3.6%, respectively. The P intake was on average 6.6 kg P/sow/year, 0.21 kg P/weaner and 0.91 kg P/grower-finisher pig with a CV of 10.7%, 7.4% and 4.4%, respectively. The variation ex-storage was only partly explained by CV in intake of N and P. The gains of N and P in products were kept constant within group causing higher variation in ex-storage values than input values. In conclusion, the variations between farms in ex-storage excretion of N and P are mainly caused by differences in feed efficiency, CP and P content of the feed.

Continuous monitoring of ammonia emissions in a fully slatted, mechanically ventilated commercial finishing pig facility, Ireland

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This study aimed to determine the ammonia emission characteristics of a fully slatted and mechanically ventilated finishing pig facility. Continuous real-time measurements were carried out in a fully slatted and mechanically ventilated finishing pig facility during two monitoring periods – summer (40 days) and winter (51 days) using a Gasera photoacoustic gas analyser. Three separate and identical fully slatted finishing pig rooms with similar environmental conditions were utilised. Each room measured 12 m × 9 m and had manure tanks with a depth of 1.4 m operating under a negative-pressure ventilation system. Airflow rate in the system was measured using an anemometer. Gaseous monitoring followed the VERA test protocol and emission calculations accounted for outliers, mortality, pig numbers and incomplete monitoring days. Diets were similar during the summer period, while animals were fed different diets during winter. Model assumptions and homogeneity of variance were verified prior to applying appropriate parametric or non-parametric tests. Differences, relationships, and associations between rooms, ammonia emissions, and environmental and animal factors were assessed using the Kruskal–Wallis test, Spearman's rho and General Additive Model (GAM). On average, results showed a higher ventilation rate of 144.6 ± 17.9 m^3 pig^{-1} h^{-1} in summer compared with 58.2 ± 19.6 m^3 pig^{-1} h^{-1} in winter (mean ± SD). Differences were observed among the three rooms in winter ($p < 0.05$), whereas no differences ($p > 0.05$) were observed among the rooms during the summer period. Across the rooms, the average ammonia emission factor was 1.40± 1.3 g pig^{-1} day^{-1} in winter and 1.6 ± 1.2 g pig^{-1} day^{-1} in summer, ranging from 0.043 to 4.91 g pig^{-1} day^{-1} in summer and from 0.040 to 7.21 g pig^{-1} day^{-1} in winter. Higher emission rates were also observed during daytime across both seasons, with different peak times. A strong relationship ($p < 0.05$) was observed between ammonia emission factors and environmental parameters. In winter, all parameters were positively correlated ($p < 0.05$) except for the body weight of the pigs ($p > 0.05$), whereas in summer all parameters were positively correlated ($p < 0.05$) except for relative humidity ($p > 0.05$), despite showing a positive relationship. Ammonia concentration appears to strongly influence ammonia emissions in both seasons. Results from the GAM showed that a non-linear relationship exists between ammonia emission and environmental parameters such as temperature, relative humidity, ammonia concentration and airflow rate. In contrast, body weight showed a linear but significant relationship with ammonia emissions. A good agreement was also found between the predicted and measured ammonia emission across the three rooms and seasons, with room A having the best model fit, followed by rooms C and B. Results from this study enhanced the understanding of environmental drivers of ammonia emissions and the emission characteristics of mechanically ventilated, fully slatted finishing pig facilities, supporting the application of targeted mitigation strategies and improvement of emission inventories for the Irish pig production sector.

Integrated nutritional and housing strategies to improve nitrogen utilization efficiency and mitigate ammonia emissions in pig production

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Ammonia (NH3) emissions from pig production pose a major environmental threat, contributing to eutrophication of ecosystems, soil acidification, and the formation of secondary particulate matter (PM2.5), with ecological and health consequences. Inefficient nitrogen utilization results in substantial urinary nitrogen excretion, which is the major precursor of NH3 volatilization, making the pig sector one of the largest agricultural sources of reactive nitrogen loss. It was hypothesized that combining low crude-protein diets supplemented with synthetic amino acids with modified housing systems would improve nitrogen utilization efficiency and significantly reduce ammonia emissions in grow-finish pigs without compromising growth performance. The objective of the study was to evaluate the combined effects of reduced crude-protein (CP) diets supplemented with synthetic amino acids and optimal housing systems on nitrogen utilization efficiency (NUE) and NH3 emissions in grow-finish pigs. A total of 144 grow-finish pigs were allocated to four treatment groups in a randomized complete block design based on initial body weight, with six replicate pens per treatment and six pigs per pen. Dietary interventions were implemented in 10 g/kg increments of crude protein reduction, with essential amino acids adequately supplemented with lysine, methionine, threonine, and tryptophan to maintain animal performance. Modifications to housing included a urine-feces separation system with V-shaped slurry scrapers and increased slurry removal frequency to limit substrate availability for NH3 formation. Photoacoustic infrared spectroscopy was used to measure ammonia concentration and estimate emission flux, while nitrogen mass balance, slurry pH, and total ammoniacal nitrogen (TAN) were monitored during the trial. Data were analyzed in R using a linear mixed-effects model with treatment as a fixed effect, block and pen as random effects, and significance was declared at $P < 0.05$. Findings showed that a 10 g/kg decrease in CP resulted in a 10% decrease in NH3 emissions, and dietary fiber intake lowered slurry pH by 0.4 to 0.6 units, thereby inhibiting volatilization by 38% ($P < 0.05$). The combined housing and nutritional approach produced a cumulative NH3 reduction of 50-54% compared with conventional systems, and growth performance was not significantly affected ($P > 0.05$). Such savings are reflected in quantifiable reductions in reactive nitrogen deposition in local ecosystems and help meet farm-level national and EU emission limits, indicating that precision nutrition and housing innovation can be a scalable, cost-effective route towards sustainable pig production.

Low-protein diets with graded essential amino acid supplementation as a strategy to improve nitrogen utilisation and reduce ammonia production in growing-finishing pigs reared under contrasting hygienic conditions

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Nitrogen (N) losses from pig production are a major environmental concern, contributing to approximately 15% of global livestock ammonia (NH3) emissions. Reducing dietary crude protein (CP), and thus N intake, is an established strategy to improve N efficiency and decrease N losses. Adequate essential amino acid (EAA) supplementation may further optimize N utilisation without compromising growth. This study evaluated the effects of low-protein diets with graded EAA supplementation on N utilisation and in vitro NH3 production in slurries of growing-finishing pigs reared under contrasting hygienic conditions. Forty-eight Swiss Large White pigs (females and castrated males; initial BW 20 ± 2.2 kg) were assigned to three isoenergetic low-protein diets, all formulated with CP reduced by 20% relative to the Swiss feeding recommendations for pigs (grower: 124 g/kg CP; finisher: 100 g/kg CP), but differing in the level of supplemental digestible EAA (Lys, Met, Thr, Trp, Val): at 80% (LP), 100% (LP+), or 120% (LP++) of requirements. To assess hygiene effects, LP pigs (n = 24) were equally divided between good hygiene (daily manure removal) and poor hygiene (weekly manure removal) conditions, while LP+ and LP++ pigs (n = 12 per group) were housed exclusively under poor hygiene. This resulted in four experimental groups: LP good hygiene, LP poor hygiene, LP+ poor hygiene, and LP++ poor hygiene. Weekly BW, feed intake (FI), and carcass composition were assessed for each pig. Faecal and urinary spot samples were collected during the grower and finisher periods. In vitro NH3 production was measured on reconstituted slurry obtained from the faecal and urinary spot samples using a dynamic chamber system. Data were analysed using linear models (R, v4.4.1), with hygiene effects assessed within LP pigs (LP good hygiene vs. LP poor hygiene) and dietary effects assessed among pigs under poor hygiene (LP poor hygiene vs. LP+ poor hygiene vs. LP++ poor hygiene). Hygiene did not affect any of the traits considered. Over the entire grower-finisher period, average daily gain did not statistically differ among dietary treatments. However, EAA supplementation significantly reduced total FI, with LP+ and LP++ pigs spending on average 3.5 fewer days on feed and consuming 4.2% less feed than LP pigs ($P \leq 0.018$), resulting in a 4.1% higher feed efficiency ($P = 0.010$). Despite a 2.9% lower CP intake, LP+ and LP++ pigs maintained similar carcass protein content compared with LP pigs, leading to a 6.5% higher N deposition efficiency ($P \leq 0.015$). Faecal N concentration did not differ among treatments, whereas urinary N concentration tended to decrease in LP+ and LP++ pigs, resulting in a significantly lower urinary N:creatinine ratio ($P = 0.015$). Total in vitro NH3 production was reduced by EAA supplementation and was on average 38.2% lower in slurry from LP+ and LP++ pigs compared with LP pigs, with NH3 peak values also 38.0% lower and peak occurrence delayed by 43.3 h ($P \leq 0.034$). Importantly, no differences were detected between LP+ and LP++ pigs for any of the traits evaluated, indicating that EAA supplementation above requirement levels did not confer additional benefits. Overall, reducing dietary CP combined with adequate EAA supplementation improved N utilisation and markedly reduced in vitro NH3 production without impairing growth. These findings highlight that meeting minimum EAA requirements in low-protein diets is sufficient to enhance production efficiency and mitigate nitrogen losses, even under poor hygienic conditions.

A controlled medium scale open circuit chamber approach to quantify the effect of crude protein and lysine supply on nitrogen efficiency and ammonia emissions in pigs

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Improving efficiency of nitrogen (N) utilization is essential to reduce N excretion and ammonia (NH_3) emission in pig production. Many nutritional mitigation strategies are first tested under laboratory conditions, but validation at barn scale remains challenging due to large environmental variation. To bridge this gap, this study used medium-scale open circuit chambers that allow controlled, replicated measurements on small groups of pigs, providing a unique experimental scale between lab-based assays and full commercial housing. We tested a key nutritional strategy: reducing crude protein (CP) while maintaining ideal amino acid balance. We investigated the effects of CP reduction and standardized ileal digestible lysine (SID Lys) supply in growing–finishing pigs on growth performance, N balance and NH_3 emission. Two crossover trials were conducted in six open circuit chambers, each housing five barrows. In Trial 1, pigs (initial BW 68 kg) received low (12 %) or high (16 %) CP diets, both with 8.5 g/kg SID Lys. In Trial 2, pigs (initial BW 42 kg) received diets with an adequate (8.3 g/kg) and deficient (7.0 g/kg) SID Lys level at constant CP (15 %). Each experimental period consisted of one week of adaptation to diets followed by three weeks in the chambers. Diets were switched for each group in the second period with the same group of pigs housed in the same chamber during both periods. Slurry was continuously collected, BW and feed intake were recorded when entering and leaving the chambers, and NH_3 concentration was monitored sequentially for 15 min in outlet air of each chamber during the final week. Nitrogen efficiency was calculated as the proportion of dietary N retained in the body based on start and end body weight. Faecal N excretion was estimated by the dietary N intake and an estimated value for CP digestibility (75 %), whereas urinary N excretion was estimated from N intake, faecal N excretion and estimated N-retention in the body. Chamber was considered as experimental unit, and data were analysed using ANOVA with dietary treatment, period and their interaction (if P value for interaction <0.20) as fixed factors and chamber as random factor for repeated measurements. Preliminary results show that in Trial 1, reducing CP by 4 % points did not affect daily gain or feed intake. However, a period-dependent effect on feed conversion ratio (FCR) was observed, with a significant lower FCR (2.43 versus 2.72 g/g) in the pigs receiving the high CP diet compared to the low CP diet in the first period, but no significant effect of dietary treatments was observed in the second period. Across both periods, lowering CP markedly improved N utilization, resulting in a 35 % reduction in total N excretion, a 24 % increase in N efficiency, and a 45 % reduction in NH_3 in the air compared to the high CP diet. In Trial 2, no diet × period interactions were observed. Adequate SID Lys supply improved animal performance, increasing daily gain by 10 %, while reducing FCR by 8 % compared with the Lys-deficient diet. Lys deficiency impaired N utilization, leading to 6 % higher total N excretion, 9 % lower N efficiency, and a 33 % increase in NH_3 concentrations. These trials demonstrate that reducing CP, while maintaining an adequate amino acid balance, is an effective strategy to mitigate NH_3 emission in growing–finishing pigs. The open circuit chamber set-up proves particularly valuable to quantify differences in excretion and emission of N at a scale that bridges the gap between experimental controlled and practical housing conditions.

Effects of dietary lysine supply on nitrogen utilisation in organic growing–finishing pigs

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The lack of nutrient guidelines tailored to organic pigs forces pig producers to rely on conventional requirements, often resulting in excessive crude protein (CP) intake and increased nitrogen (N) excretion when meeting essential amino acid standards. This study aimed to evaluate the physiologically optimal supply of standardised ileal digestible (SID) lysine for organic growing–finishing pigs by quantifying N utilisation across compound feed differing in SID lysine content. We hypothesised that N utilisation would be low at insufficient SID lysine levels, increase toward an optimal supply, and decline again at excessive lysine levels due to oversupply. Thirty female DLY pigs (average weight 30.9 ±1.6 kg) were assigned to one of five compound feeds with increasing SID lysine concentrations (g/kg; Lys 6.1, Lys 6.5, Lys 6.9, Lys 7.3, and Lys 7.7). Lysine levels were increased by raising overall CP content while maintaining a constant SID lysine: CP ratio. To comply with organic production principles, pigs had ad libitum access to grass–clover silage (GCS). Two three-day balance trials were conducted: trial 1 at an average pig weight of 57.2 kg, and trial 2 at 99.6 kg. During each trial, urine was collected via catheter, faeces was collected quantitatively, and individual intake of compound feed and GCS was recorded. Blood samples were taken from the jugular vein at the end of each trial and analysed for urea and creatinine. Nitrogen in urine and faeces could not be attributed separately to compound feed or GCS, so in the calculations, N from both sources was treated as a single pool for intake as well as for urinary and faecal excretions. Data were analysed using PROC GLIMMIX in SAS. Three-day GCS intake ranged from 863-1479 g in trial 1 and 1330-2252 g in trial 2 and was unaffected by compound lysine level, indicating no compensatory roughage intake. In trial 1, increasing SID lysine elevated N intake ($P = 0.027$) and urinary N output ($P = 0.018$), resulting in an overall diet effect on N balance ($P = 0.022$). However, N digestibility (78.9–81.5 %) and N retention (68.5–73.3 % of digested N) were unaffected. Plasma urea/creatinine ratio differed among diets ($P = 0.044$), but post-hoc comparisons detected no significant pairwise differences. In trial 2, three-day N intake increased from 222 g in the Lys 6.5 group to 268 g in the Lys 7.7 group ($P < 0.001$). Faecal N excretion was unaffected, but the three-day urinary N output rose from 58.9 g in the 6.1 Lys group to 92.1 g in the Lys 7.7 group ($P < 0.01$). Although overall N retention did not differ among diets, a linear decline was observed with increasing lysine ($P < 0.01$), from 68.4 % in the Lys 6.1 group to 56.9 % in the Lys 7.7 group. Plasma urea/creatinine showed a numerical increase at higher lysine levels, but only a trend towards significance ($P = 0.06$). The study demonstrates that increasing SID lysine levels in organic compound feeds elevates total N intake and urinary N excretion in growing–finishing pigs, without improving N retention. Pigs did not adjust their intake of GCS in response to dietary lysine supply, indicating limited capacity for compensatory roughage intake under organic conditions. Contrary to the hypothesised intermediate optimum, no improvement in N utilisation was observed. Within the tested range, SID lysine levels above the lowest level primarily increased N excretion rather than supporting additional protein deposition. The findings highlight the need for tailored amino acid guidelines to improve nutrient efficiency and environmental sustainability in organic pig production.

Evaluating the Role of Dietary Silicate (SiO_3) and Zinc Oxide on Growth Performance, Nutrient Utilization, Gas Emission, Blood parameter and Environmental Impact in Weaning Pigs

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Dietary zinc oxide (ZnO) is commonly used in the swine industry to reduce post-weaning diarrhea and improve growth performance in weaned pigs. However, its poor absorption in the gastrointestinal tract results in excessive zinc excretion, leading to serious environmental pollution. Consequently, alternative or complementary feed additives are being explored to sustain growth and gut health while minimizing environmental impact. Dietary silicate (SiO_3) has attracted attention for its adsorptive and buffering properties and its potential to enhance intestinal integrity and nutrient utilization. Silicate-based minerals may bind toxins, stabilize gut microflora, and reduce harmful gas emissions from manure. Therefore, this study aims to evaluate the effect of different levels of ZnO with and without SiO_3 on growth performance, nutrient digestibility, blood parameters, gas emissions, and fecal consistency in 200 [Duroc × (Yorkshire × Landrace); 28 days old; 6.41 ± 0.24 kg] weaning piglets. For a period of 6 weeks, all pigs were assigned to four dietary treatments in a 2 × 2 factorial arrangement. The dietary treatments were as follows: High and Low ZnO (HZnO, 2000ppm and LZnO, 50ppm), with (+) and without (-) 0.1% SiO_3. Each treatment has 10 replicates with 5 pigs/pen. Data was analyzed using two-way analysis of variance with GLM procedure of SAS to evaluate the effects of different ZnO levels (high vs. low), SiO_3 supplementation (with vs. without), and their interaction. Pigs fed a HZnO diet showed significantly greater body weight at the end of week 6, and higher ADG during weeks 1–6 and the overall period compared with pigs fed a LZnO diet ($P < 0.05$), with a tendency for increased ADG during week 1 and improved FCR during weeks 3–6 ($P < 0.10$). Supplementation of 0.1% SiO_3 with HZnO further increased ($P < 0.05$) ADG during weeks 3–6 and overall compared with LZnO + SiO_3. At week 6, HZnO significantly($P < 0.05$) improved nitrogen digestibility, WBC counts, lymphocyte percentage, and reduced NH_3 emissions ($P < 0.05$). Fecal consistency was improved by ZnO and silicate supplementation ($P < 0.05$). There were no interaction effects observed during the experimental period. In summary, combining dietary silicate with a high ZnO diet proved to be effective in enhancing growth performance, nutrient utilization, immune function, and mitigating gas emissions in weaning pigs. Keywords: growth performance, nutrient digestibility, weaning pigs, silicate, zinc oxide.

Reducing environmental nitrogen losses through improved nitrogen retention in weaned piglets fed humic acids

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Reducing nitrogen (N) emissions in pig husbandry systems is essential because excessive N excretion contributes to environmental challenges, such as ammonia emissions and soil- and water pollution. A common way to reduce N excretion is via reducing the protein content of the diet. However, it was hypothesized that humic acids by binding pathogenic substances[1], like endotoxins, can support intestinal health, saving N and energy for growth. Furthermore, humic acids could reduce N excretion emission through acidifying the manure and urine, thereby increasing the ratio NH_4^+:NH_3, keeping more N as NH_4^+ and thereby reducing the NH_3 gas emission. In addition, a lower pH may reduce the urease activity, resulting in less conversion of urea in the urine into NH_3 and CO_2. In this context, a digestibility trial was conducted to assess the potential of humic acids on performance, nutrient digestibility and N excretion. 48 piglets (TN70 x PIC 408 Piétrain) were weaned at 22 days of age and were housed in pairs. Piglets were evenly allocated into one of two dietary treatments based on individual weaning weight. From day 1 to 4 post-weaning all piglets received semi ad-libitum creep feed and thereafter from day 5 to 12 semi ad-libitum performance-oriented weaner diet. On day 13 the experimental period started and piglets received either a rearing diet (3.6 mm pellet) without (A) or with 0.15% humic acids (B, Humigut) until the end of the trial (day 26). Piglets were weighed individually on day 12, 19 and 26 of the trial. The first week piglets received the diets semi ad-libitum to adapt and no measurements were taken. From day 20 to 26 piglets were fed restrictedly based on registered weights on day 19. Feed intake was measured per pen, faecal samples were collected twice per day and urine samples once per day between day 22 to 26. On day 22 and 23 the faeces and urine were analysed separately to determine nutrient digestibility. On day 24 to 26 the faeces and urine were collected separately but were mixed into a slurry and stored uncovered to mimic the manure pit to measure N excretion[2]. On day 26 a pooled sample was taken for analysis. All data were statistically analyzed using R Studio with an ANCOVA model, using weight on day 12 as a covariate. The inclusion of humic acids resulted in improved growth (4.0 vs. 4.5 kg/piglet; P=0.003) between day 13-26 and therefore a significantly higher weight on day 26 (11.2 vs. 11.7 kg/piglet; P=0.004). Nitrogen absorption was similar between treatment groups, but N retention was significantly higher for group B (70.9 vs. 74.7%; P=0.02). Increased N utilization by the pig implies that a smaller proportion of N is excreted via the manure. The manure pH was significantly lower in group B (6.6 vs. 6.2; P=0.01). It is known that the acidification of pig manure (pH 5-6) could reduce urease activity[3], which decreases NH_4^+ formation. Although the NH_4^+ content in the slurry of both groups was similar, piglets in group B significantly produced less slurry (734 vs 575 g/pig/day; P=0.03). Therefore, the calculated NH_4^+ excretion per pig per day was significantly reduced in group B (1968 vs 1640 mg/pig/day; P=0.02). There was no difference between fat- and dry matter absorption between treatment groups. The results of this trial showed that the addition of humic acids results in a significantly higher N retention, which proves that more N is used for growth rather than being excreted as waste. In addition, less N would be available for conversion to ammonium, which reduces environmental emissions. On that account, humic acids could contribute to more sustainable pig husbandry.

Relationship between nitrogen and phosphorus intake during pig fattening and the CO_2 footprint per kilogram of live weight.

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Germany aims to reach climate neutrality by 2045. In conventional pig production systems, feed is typically the dominant emission source, contributing roughly half to more than 60% of total greenhouse gas output. Consequently, nutrient use during fattening plays an important role. This study therefore aimed to describe the relationship between nitrogen (N) and phosphorus (P) intake during pig fattening and the CO_2 footprint per kilogram of live weight. The analysis was based on data from a representative sample of 21 fattening groups (grower to finisher) from a total of 13 conventional pig finishing farms located in northwestern Germany. All farms were connected to the same piglet production system and operated under similar management practices. The recorded data included the raw components used in the compound feeds, their average consumption per animal, and the contents of N (crude protein/6.25) and P. In addition, performance data such as live weight at entry and exit were documented. The life cycle assessment was carried out using the software SimaPro Craft, version 10.2.0.2, with the GFLI database. The exact input of N and P was calculated by multiplying the content of the feed by the amount of feed consumed. Emissions from the barn and from outdoor manure storage with a natural crust cover were calculated according to the literature. The calculated nutrient excretion of N, P, and potassium are contained in the slurry. As an organic fertilizer, slurry can reduce the need for synthetic fertilizers in crop production. The production of synthetic fertilizers is highly energy intensive. In the pig production model used in this study, the amount of synthetic fertilizer that can be replaced by applying the produced slurry to cropland was credited. Electricity consumption for the production of 1 kg of feed was set at 0.02 kWh. The assessment was carried out based on a cradle-to-farm model with a functional unit of 1 kg of liveweight pig. The statistical analysis was performed using SAS Enterprise Guide (version 7.1, SAS Institute Inc., Cary, NC, USA). Normal data distribution was assessed using the Kolmogorov–Smirnov test. Subsequently, the Spearman correlation coefficient was calculated. N and P intake were not normally distributed, whereas the CO_2 footprint data were. Higher total N intake per kg of weight gain during fattening correlated positively with the carbon footprint per kg of live weight (Spearman's $r = 0.61$, $p = 0.003$). No clear correlation was found for P intake ($r = 0.25$, $p = 0.27$). The raw materials with the highest P contents were wheat bran (13 g/kg DM) and rapeseed meal (12 g/kg DM), while soybean meal (7 g/kg DM) and cereals (4 g/kg DM) contained less. The soybean meal included was not certified as deforestationfree, which increased the calculated carbon footprint. Today, soybean meal used in Germany is typically from production without deforestation. If the land use change from this study would be excluded, the correlations would become more pronounced (N: $r = 0.74$, $p < 0.001$; P: $r = 0.56$, $p = 0.008$). In conclusion, N intake during the fattening phase appears to be a relevant driver of the carbon footprint per kg of live weight, whereas P intake showed not such a clear association. These findings confirm the importance of optimizing N efficiency in livestock production to mitigate environmental impacts.

Feeding the balance: how diet optimisation goals affect nitrogen and phosphorus inputs and outputs in Swiss grower-finisher pigs

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The Swiss agricultural system uses two annual nutrient balances to assess nitrogen (N) and phosphorus (P) flows. The first balance (Balance 1) compares national agricultural inputs and outputs. It currently shows a surplus that must be reduced by 15% for N and 20% for P by 2030. The swine sector can contribute by reducing imports of N- and P-rich feedstuffs such as soybean meal and mineral P, which account for 47% of imported N and 56% of imported P in animal feed. Greater use of domestic resources should therefore be promoted. The second balance (Balance 2) is calculated at farm level and compares crop fertilisation requirements with fertiliser use. To avoid surpluses, i.e. excessive N and/or P excretion, one strategy is to minimise dietary crude protein (CP) and/or P content. The Swiss Confederation already enforces a maximum ratio of CP to digestible energy (DE) in pig feed which must not exceed 10.5 g CP/MJ DE throughout the fattening period. This study evaluated seven scenarios for optimising least-cost two-phase diets for growing–finishing pigs in order to assess: (1) how reducing imported N and P sources (Balance 1) affects N and P excretions (Balance 2), and (2) how maximising reductions in N and P excretions (Balance 2) influences the use of imported N and P sources (Balance 1). Seven least-cost optimisation scenarios (SCEN) were tested for grower (40 kg BW) and finisher (80 kg BW) diets containing 13.7 MJ DE/kg and a fixed calcium to digestible P ratio of 2.8. All diets met the Swiss requirements for the nine essential amino acids and digestible P. SCEN 0 represents a compromise optimisation limiting both input and excretion. SCEN 1–3 prioritise reducing N and P inputs (SCEN 1), or N input alone (SCEN 2) or P input alone (SCEN 3). SCEN 4–6 prioritise reducing excretions by imposing minimum CP and P levels (SCEN 4), or minimum CP (SCEN 5) or minimum P (SCEN 6), without restrictions on ingredient origin. The N and P intake per animal was calculated based on dietary CP and P levels, assuming feed intake of 90 kg in the growing and 125 kg in the finishing period. The share of N and P derived from imported feedstuffs was also determined. N and P excretions were estimated for 80 kg weight gain, assuming fixed retentions of 2.08 kg N and 432 g P. Results were expressed relative to SCEN 0. Compared with SCEN 0, input-oriented SCEN (1–3) reduced N from non-Swiss feedstuffs by 23–55% and mineral P use by 10–27%. However, they increased P excretion by 13–16% and had little effect on N excretion due to the reached CP/DE limit. Feed costs rose by 3% in SCEN 2 due to higher inclusion of synthetic amino acids. Excretion-oriented SCEN (4–6) reduced N excretion by 3–17% and P excretion by 25–30%, but markedly increased reliance on non-Swiss feedstuffs (+33–77% N from soybean meal and potato protein; +65–100% P from mineral P). When the objective was to reduce both N and P excretion (SCEN 4), or N alone (SCEN 5), feed costs increased by 5–9% as lower CP levels required greater use of synthetic amino acids and potato protein. The study highlights the high sensitivity of N and P balances to optimisation constraints. Prioritising either input reduction or excretion reduction generates substantial trade-offs between nutrient flows, costs and ingredient origin. Reducing N and P inputs (Balance 1) is possible but negatively affects Balance 2, particularly P excretion, as the ingredients used (rapeseed or sunflower meal and milling by-products) are rich in P. Similarly, formulations targeting lower N and P excretions (Balance 2) worsen Balance 1 by promoting the use of feedstuffs rich in N and P (soybean meal, potato protein and mineral P).

Impact of swine influenza on greenhouse gas (GHG) emissions on Japanese commercial farms

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Reducing GHG emissions is a critical social goal in achieving a sustainable society. Pig production contributes to GHG emissions through various processes, primarily from manure management, animal housing, and feed production and transportation. GHG emissions of pig production are notably influenced by farm productivity. Swine influenza is a respiratory disease caused by influenza A viruses of swine (IAV-S), which is one of the major respiratory pathogens affecting pigs in large parts of the world. Infection results in clinical signs of fever and coughing, which leads to decreasing productivity and substantial financial losses. However, no study has yet been conducted in Japan to investigate the extent to which IAV-S affects GHG emissions. Therefore, this study aims to investigate the impact of the presence or absence of swine influenza on GHG emissions from pig farms. This study collected data from 9 farrow-to-finish commercial farms in Japan. Active surveillance was performed in July 2024, October 2024, and January 2025 to collect nasal swab samples that were randomly collected from 30 weaned pigs by veterinarians on each farm. The samples were examined for the presence of IAV-S by real-time RT-PCR. Farms with at least one IAV-S positive sample in all three surveillance rounds (n = 4) were defined as ”high IAV-S farms,” while the remaining farms testing positive in fewer than three rounds (n = 5) were defined as ”low IAV-S farms.” Using the calculated GHG emissions as outputs, we compared the emissions between high and low IAV-S farms for finishing section. To assess the GHG emissions in each process, productivity indicators were obtained directly from the surveyed farms. For manure management, GHG emissions was calculated by four processes: CH_4 and N□O generated from excreta, indirect N□O emissions from NH□ during feeding, and CO_2 from fuel and electricity associated with manure management. For animal housing, GHG emissions were calculated by fuel and electricity-derived CO_2 associated with animal housing in the barn and enteric CH_4. The functional unit in finishing section was 1 kg of carcass weight of fattening pig. In the finishing section, high IAV-S farms showed numerically higher emissions across all processes: feed production and transport (2092.17 ± 162.07 vs. 1951.77 ± 119.44 g CO_2e), animal housing (690.04 ± 60.91 vs. 647.28 ± 30.46 g CO_2e), and manure management (1375.01 ± 121.38 vs. 1289.79 ± 60.70 g CO_2e), with a total of 4157.2 ± 208.9 vs. 3888.8 ± 202.8 g CO_2e/kg carcass weight. This study assessed the impact of swine influenza on GHG emissions across nine Japanese farms. Despite the small sample size, GHG emissions were consistently higher on high IAV-S farms than low IAV-S farms in finishing section. These findings demonstrate that swine influenza prevalence negatively impacts environmental sustainability by increasing GHG emissions per unit of production.

Evaluation on low crude protein diet with protease supplementation on growth performance, fecal score, backfat thickness and lean meat percentage, nutrient digestibility, meat quality, and carcass grade in finishing pigs

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The purpose of this study was to evaluate the effects of protease supplementation in a low crude protein (CP) diet on growth performance, fecal score, backfat thickness, lean meat percentage (LMP), nutrient digestibility, meat quality, and carcass grade in finishing pigs. A total of 96 crossbred pigs [Landrace x Yorkshire) x Duroc] with an initial body weight (BW) of 59.75 kg were used in a 9-week trial. Pigs were randomly assigned to one of three dietary treatments, with eight replicates per treatment and four pigs per pen in a randomized complete block design. The dietary treatments were positive control (PC as a typical finishing diet based on corn and soybean meal); negative control (NC) (low crude protein diet); and NCP (low CP + 0.025% protease). Data was analyzed as a randomized complete design using one-way ANOVA in the GLM procedure of SAS (SAS Institute, Cary, NC, USA). $P < 0.05$ was set as significant. Protease supplementation had no significant effects ($p > 0.05$) on BW, average daily gain (ADG), average daily feed intake (ADFI), and feed conversion ratio (FCR) at week 5. However, FCR tended to increase ($p = 0.056$) at week 9, while BW, ADG, and ADFI remained unchanged throughout the entire trial. Fecal score and lean meat percentage (LMP) were not significantly affected by protease supplementation. However, nitrogen (N) digestibility was increased significantly ($p = 0.0215$) in pigs fed the NCP diet. Meat quality traits, including pH, water holding capacity (WHC), longissimus muscle area, meat color, cooking loss, drip loss, and sensory evaluation, were not affected ($p > 0.05$) by protease supplementation. Nonetheless, there was a tendency for differences in day 1 drip loss ($p = 0.060$) and marbling score ($p = 0.091$) in the protease-supplemented pigs. Furthermore, carcass grade and backfat thickness were not significantly affected by protease inclusion. In summary, protease supplementation in a low crude protein diet enhanced nitrogen digestibility and showed a tendency to improve feed efficiency in finishing pigs without adversely affecting growth performance, fecal score, carcass characteristics, or meat quality. These findings indicate that dietary protease can support efficient protein utilization and allow for a reduction in dietary crude protein and soybean meal inclusion in finishing pig diets, contributing to more sustainable and cost-effective swine production systems. Keywords: Protease, low crude protein, growth performance, nutrient digestibility, meat quality.

Effects of dietary silicate supplementation with varying levels of proteins on growth performance, nutrient digestibility, gas emission, blood profile and fecal score in weaning pigs

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Weaning is a critical and stressful phase in pig production, often resulting in reduced growth performance, impaired nutrient utilization, and compromised gut health. Although adequate dietary protein is essential for growth, excessive protein intake can increase nitrogen excretion and harmful gas emissions. Silicate (SiO_3) has emerged as a functional feed additive with potential benefits for nutrient digestibility, intestinal health, and ammonia reduction; however, its combined effects with varying dietary protein levels in weaning pigs remain limited. Therefore, the objective of this study was to evaluate the effects of dietary silicate supplementation under different protein levels on growth performance, nutrient digestibility, gas emissions, blood profiles, and fecal characteristics in weaning pigs. A total of 200 crossbred weaning pigs ([Yorkshire × Landrace] × Duroc, 21 d old with an initial body weight (BW) of 6.47 kg) were randomly allotted to 1 of 4 dietary treatments based on the BW and sex and in a 2 × 2 factorial arrangement. Each treatment has ten replicates with (5 pigs per pen [2 barrows and 3 gilts]. Data was analyzed using two-way analysis of variance with GLM procedure of SAS to evaluate the effects of different crude protein levels (high vs. low), SiO_3 supplementation (with vs. without), and their interaction. The dietary treatments were as follows: High Crude Protein (HCP) and Low Crude Protein (LCP), with (+) and without (-) 1% SiO_3. Pigs fed an HCP + SiO_3 diet showed significantly greater average daily gain (ADG) during weeks 1–6 and over the entire experimental period ($P < 0.05$). Also, it resulted in increased nitrogen digestibility and reduced fecal ammonia (NH_3) emissions ($P < 0.05$). Compared with pigs fed LCP diets with or without SiO_3, those receiving the HCP + SiO_3 diet exhibited higher white blood cell counts ($P < 0.05$) and a tendency toward increased lymphocyte percentages ($P < 0.10$). No interaction effects between dietary protein level and silicate supplementation were observed for any measured parameters. In conclusion, dietary supplementation with 0.1% silicate improved growth performance, nitrogen utilization, immune response, and intestinal health and reduced fecal ammonia emissions in weaning pigs, independent of dietary protein level. Keywords: growth performance, nutrient digestibility, weaning pigs, silicate, protein

Individual and Combined Supplementation of Microbial Phytase and β-Mannanase in Growing Pigs: Impacts on Growth Performance, Nutrient Digestibility, and Fecal Scores

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In modern pig production, enhancing growth efficiency and nutrient utilization while minimizing costs and environmental impact is a key priority. Nevertheless, digestive inefficiency and nutrient loss continue to challenge sustainable production. Therefore, the objective of this study was to assess the effects of single and combined effect of microbial phytase and β-mannanase on growth performance, nutrient digestibility, and fecal score in growing pigs. For a period of 35 days, a total of 128 crossbred pigs [(Landrace × Yorkshire) × Duroc] with an initial body weight of approximately 29.61 ± 1.57 kg were randomly assigned to one of four dietary treatments: (1) CON, a basal diet without enzyme supplementation; (2) TRT1, the basal diet supplemented with 500 U/kg microbial phytase; (3) TRT2, the basal diet supplemented with 1600 U/kg β-mannanase (4) TRT3, the basal diet supplemented with 1600 U/kg β-mannanase + 500 U/kg microbial phytase. Each treatment included 8 replicates with 2 barrows and 2 gilts per pen. Data was analyzed as a randomized complete design using one-way ANOVA in the GLM procedure of SAS (SAS Institute, Cary, NC, USA). $P < 0.05$ was set as significant. The inclusion of microbial phytase and β-mannanase in pigs diet had significantly improved ($P< 0.05$) average daily gain (ADG) and feed conversion ratio (FCR) during days 0–5. From days 5–35 and throughout the entire experimental period, all enzyme-supplemented groups exhibited significantly ($P < 0.05$) higher ADG and lower FCR compared with the CON group. Similarly, nutrient digestibility of dry matter, nitrogen, and energy were significantly ($P< 0.05$) increased in pigs fed microbial phytase and β-mannanase supplementation. No significant differences were observed in fecal scores among the treatment groups ($P > 0.05$). Collectively, these results suggest that the combined use of microbial phytase and β-mannanase is an effective strategy to enhance growth performance in growing pigs. Keywords: microbial phytase; β-mannanase; growing pigs; growth performance; nutrient digestibility; fecal score

Feasibility and repeatability of standard protocol to assess animal welfare

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Animal welfare is assessed throughout the animal's life, including on-farm, during transport and at slaughter. Welfare protocols have therefore been developed in several research projects, such as Welfare Quality®, AWIN, ClassyFarm, and EBENE. To obtain a clear overview of the animal welfare's progress in Europe, the internal project (SOA9) of the European Partnership on Animal Health and Welfare (EUPAHW) is developing a knowledge platform compiling all these elements. Within SOA9, the different welfare protocols used at the European scale have been reviewed to harmonize and standardize animal welfare protocols focusing on 60 indicators across 9 livestock species and production categories, i.e., dairy cattle, pig, sheep, goat, broiler chicken, laying hens, turkey, rabbit and fish. In addition, a specific study is currently being set in poultry to assess feasibility and repeatability of the standardized protocols, where there is a gap of knowledge. For laying hens, the following indicators have been assessed on-farm using the transect method: feather damage, beak condition, foot damage, toe damage, and wounds. On the other hand, plumage cleanliness, breast blisters, and footpad dermatitis have been assessed in broiler chickens at the slaughterhouse. Feasibility of protocols has been assessed in at least three different rearing systems (e.g. cage, floor, aviary for laying hens) using an assessment methodology that considers the following aspects: specific equipment required, automatization of assessment, time required, animal handling, training time, and doable of the slaughter line (if the assessment is carried out at the slaughterhouse). To assess repeatability, the protocols used to evaluate the above-mentioned indicators have been carried out by three observers (inter-observer repeatability) and in three rounds (intra-observer repeatability). The results will feed the EUPAHW knowledge platform by displaying repeatability, feasibility, and the level of effort required for implementation for each indicator and its protocol.

Animal Welfare Index – A new on-farm welfare assessment method

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On European level, one of the biggest organizations responsible for the control of food production chains is QS® – Quality Scheme for food (QS Qualität und Sicherheit GmbH, 53177 Bonn, Germany). Within this scheme, health and welfare of livestock are mainly rated based on observations made at the slaughterhouse. These observations are determined by a protocol named Animal Welfare Index. The index is classified into four primary categories: 1) respiratory health, 2) organ health, 3) limb health, and 4) integrity. The primary categories are furthermore divided into subclasses identifying the affected organs or the pathologies: 1) lung, pleura, pericardium, 2) liver, intestines, 3) joint inflammation, bursitis, 4) skin, ear, tail, and chasing marks. Subsequently, the rating is converted into values using a scale ranging from 0 to 100, with 0 representing the lowest score and 100 the highest attainable score. The aim of this study is to propose an additional index that is based on scores documented at farm level and focused on living animals. A thorough examination of the production process has led to the identification of three candidates time points where the rating could be implemented: weaning; the end of the rearing process; and day 80 of the fattening period. For the development of the farm index, the primary categories were left unaltered, while the subclasses underwent modifications to ensure their adaptability on farm level. The subclasses include new parameters to be measured on living animals like: 1) nasal discharge, chough, sneezing, cardiovascular problems, 2) diarrhea, 3) lameness, arthritis, hoof changes, 4) old/fresh ear injury, loss of tail length, tail tip necrosis, and old/fresh tail wound. For testing, the assessments were conducted on four farms, which were found to comply the production standards 1 to 4 of the German "Haltungsform (HF)". These housing standards range progressively from conventional pig farming (HF1) to an enhanced system that provides 100% more space per animal and continuous access to outdoor areas (HF4). Preliminary results demonstrated congruence with marginal differences between the ratings of the standard slaughterhouse index and the novel on farm index for respiratory health (91 vs. 98), organ health (95 vs. 99), and limb health (95 vs. 92). Nevertheless, discrepancies between the indices were found for the primary category of integrity (97 vs. 48). The on-farm index includes a higher percentage of damages compared to the slaughterhouse index. One of the advantages of the on-farm index is the ability to facilitate management decisions within the farm and at the slaughterhouse. However, for monitoring of integrity, particularly tail lesions in the context of tail biting, the on-farm index reveals a more detailed picture to improve husbandry and management conditions regarding animal welfare. The project is supported by funds of the Federal Ministry of Agriculture, Food and Regional Identify (BMLEH) based on a decision of the Parliament of the Federal Republic of Germany. The Federal Office for Agriculture and Food (BLE) provides coordinating support for future farms and future regions as funding organization, grant number 2823ZR016.

New welfare assessment protocol for dairy cattle herds

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The present study developed a new set of measurements for assessing animal welfare in dairy herds. The protocol created reflects five dimensions of welfare: nutrition, health, environment, behaviour, and emotional state. It covers all animal groups on a dairy farm: calves, youngstock, and dairy cows. Our goal was to create a welfare assessment protocol scientifically valid yet practical and time-efficient. We aimed for a procedure that can be implemented widely and is compatible with the existing quality assurance systems in Finland. Therefore, it will be useful in promoting welfare improvements in high proportion of dairy farms in the future. The development process consisted of a literature review to identify potential measurements, followed by testing feasibility and reliability and estimating required sample sizes. The protocol was tested on dairy farms in three phases. Measurements showing low inter-observer reliability or limited practicality were refined or removed. Sample sizes for animal-based measurements were defined using field data and published values in scientific literature. During development, an understanding of welfare as the animal's own subjective experience was emphasized; therefore, animal-based measurements were prioritized. However, to realistically achieve scalability, on-farm assessment of a typical size of a Finnish dairy herd (40 to 200 cows) should not take more than half of a working day. Consequently, some of the most time-consuming animal-based measurements were replaced with reliable alternatives of resource- or management-based measurements, when satisfactory options were available. Also, the protocol was designed to interfere with farm routines as little as possible. In addition to measurements conducted on-farm, we incorporated into the protocol several database-derived indicators (for example mortality, incidence of mastitis, structural restrictions, adequacy of claw trimming). This was possible, because in Finland, veterinarians, advisors, and farmers routinely collect structured data relevant to welfare. Data are sourced from the Naseva herd health and welfare register, the national cattle register, and Dairy Herd Improvement records. Moreover, a complementary questionnaire for farmers was included. It covers measurements that cannot be obtained from databases or farm-level measurements (for example calf feeding practices, outdoor access, and using pain relief). The final welfare assessment protocol includes 44 measurements: 17 measurements conducted on farm, 9 database-derived variables, and 18 questions for farmers. Unlike many earlier protocols, this one covers pre-weaned calves and youngstock. We developed some new measurements specifically for them, also, measurements that have earlier been developed for adult animals but were suitable for calves and youngstock are used. The number of measurements vary between animal groups. Pre-weaned calves and dairy cows are assessed individually (e.g. body condition score, skin lesions, symptoms of disease), whereas youngstock are assessed at group level from outside pens, because of safety and reliability. The welfare assessment protocol system is designed to be dynamic, allowing updates as welfare research and technology continuously advance. Future developments may include additional measurements, particularly those addressing positive welfare. Work to create an aggregation method for generating welfare indices is ongoing.

Potential of barn climate parameters as iceberg indicators in welfare assessment and their relationships with productivity in fattening pigs

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Welfare assessment on-farm by animal-based measures remains time-consuming and therewith lacks feasibility. The use of post-mortem indicators at the slaughterhouse is also problematic, as these data are retrospective in nature. The question thus arises whether currently applied animal-based indicators could be partially substituted by barn climate data. These can be continuously recorded by widely available and affordable digital devices. This study investigates the potential of barn CO_2 concentration (CO2), relative humidity (HUM) and the temperature-humidity index (THI) as potential iceberg indicators for welfare assessment in fattening pigs. Climate sensor data and weekly assessed on-farm indicators were collected on 2 conventional German pig farms with forced ventilation. Post-mortem indicators, including performance data, were recorded at slaughter. Data were available for 387 pigs in all. To capture short- and medium-term barn climate effects (potentially reflected in on-farm animal-based indicators), CO_2, HUM and THI were summarized by their mean values on the respective assessment morning and by weekly mean, minimum, maximum and cumulative values (loads) for the preceding week. To capture long-term effects (potentially reflected in post-mortem indicators), this was carried out for three periods: 4 and 8 weeks before slaughter and the entire fattening period. Logistic regression models were calculated with the dependent variable being the respective animal welfare indicator (on-farm (e.g. sneezing, tail lesions) or post-mortem (e.g. pneumonia, pleuritis) and the independent variables the respective climate indicator and the pen. For the performance data analysis, the dependent variable was the respective performance parameter obtained from the slaughterhouse via AutoFOM (e.g. carcass weight, lean meat percentage) and the independent variables were the respective climate indicator and the age at slaughter. The analysis for the welfare indicators revealed most significant effects for HUM, particularly impacting the prevalence of ear lesions, tail lesions, fever, coughing and pneumonia. Single THI parameters affected the prevalence of ear lesions, tail lesions, sneezing and pneumonia. The CO2 parameters only impacted the prevalence of ear lesions, tail lesions and pneumonia. As for the performance indicators, multiple THI parameters significantly affected lean meat percentage, average daily weight gain and carcass weight, whereas HUM only affected lean meat percentage and carcass weight. Significant effects of CO_2 were only observed for lean meat percentage. In both farms, climate parameters only occasionally exceeded recommended thresholds. Stronger effects might have been observed under greater variability in barn climatic conditions. It is noteworthy that harmful effects of elevated CO2 were detected, although the threshold was set comparatively low at 1,500 ppm. Furthermore, our results indicate a significant impact of HUM on pig welfare and performance, suggesting that future research should place greater emphasis on this parameter. Overall, our findings confirm the potential of various barn climate parameters as iceberg indicators in pig welfare assessment.

Effect of dehorning on salivary cortisol responses in South African Bonsmara beef calves

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In South Africa, dehorning is a routine management practice performed on most beef cattle farms, either to prevent injuries to other cattle or handlers, to adhere to breed standards, or for entry into the feedlot. Dehorning is a painful and stressful procedure for calves, especially since very few farmers administer any form of medication for pain relief. Various studies observed an immediate rise in plasma cortisol levels, an indicator of stress, which can last up to six hours after dehorning. Saliva collection is a non-invasive alternative to blood sampling that incurs additional stress, especially for calves in extensive production systems. Saliva is regarded to be a good estimate of plasma cortisol following activation of the HPA axis in response to acute stress in cattle. This study aimed to measure the stress associated with dehorning in Bonsmara beef calves in a commercial setting using salivary cortisol as a biomarker. Ethical approval was obtained from the AEC of the University of Pretoria (NAS308/2025). Sixty nine Bonsmara pre-weaner calves, three to four months of age, and representing first and second parity plus cows were divided into two treatment groups: polled control group (n=35) and a dehorned group (n=34). The 34 calves were dehorned as part of routine management practices on a Bonsmara stud farm using a hot disbudding iron and treated with an aerosol ectoparasitide afterwards. Saliva samples were taken using synthetic swabs (Salivette Cortisol, Sarstedt, Germany) before dehorning, immediately after dehorning, approximately 2 hours after dehorning and the following day. Within three hours after sampling, the samples were centrifuged at 12 000 rpm for 20 minutes. Salivary cortisol concentrations were determined with a competitive cortisol enzyme immunoassay (EIA). Statistical analyses were performed in R fitting a linear mixed-effects model. Salivary cortisol concentrations differed significantly between treatments, with dehorned calves showing substantially higher cortisol levels than polled calves. Mean peak cortisol concentrations were approximately 10.8 ± 4.1 ng/ml in dehorned calves compared with 5.65 ± 2.92 ng/ml in polled calves, representing a large treatment effect (Cohen's $d \approx 1.47$). These results were similar to previous studies in cattle using plasma cortisol as a biomarker to evaluate stress. Significant effects were observed for treatment ($P < 0.001$) and sampling time ($P < 0.001$) on cortisol concentrations, with a significant treatment × time interaction ($P < 0.001$). This indicates that dehorned calves exhibited a pronounced cortisol spike following dehorning, while cortisol levels in polled calves remained relatively stable over time. Dehorning causes acute stress in beef calves, that can be mitigated by incorporating polled genetics in breeding programs.

Circulating MiRNAs as Potential Biomarkers Of Pain And Stress in Piglets Undergoing Castration

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Castration of piglets is a breeding practice performed globally, and it is usually performed without pain relief. Such a procedure raises important ethical and scientific questions regarding the welfare of piglets and the level of pain experienced by piglets. The pain assessment in animals is particularly challenging, as animals do not express pain in a way that is easily recognized or measured. Moreover, the stress caused by handling, restraint, or the mere presence of humans is often so intense that it can mask or overshadow behavioural and physiological indicators of pain. Micro ribonucleic acids (miRNAs) regulate genes involved in pain and stress pathways, are stable in biofluids, and can provide more specific information on the biological processes. Our goal was to investigate the use of circulating miRNAs as potential biomarkers of pain and stress experienced by piglets during castration and to evaluate how their expression is modulated by the administration of meloxicam in combination with isoflurane for pain relief during the castration. The study included 24 four-day-old piglets assigned to four experimental groups. The first group served as a handling control and was handled without castration. The second group underwent castration without anaesthesia or analgesia. The third group was handled following the administration of anaesthesia and analgesia but was not castrated. The fourth group underwent castration following the administration of anaesthesia and analgesia. Piglets' plasma was collected before handling or castration and 2 h, 24 h, and 48 h after handling or castration. MiRNAs were isolated from piglets' plasma using a commercially validated miRNA extraction kit. To characterize the whole profile of circulating miRNAs, a pilot next-generation sequencing (NGS) analysis was performed, and differentially expressed miRNAs (DE-miRNAs) were identified by comparing the groups at different time points. Gene ontology was subsequently conducted to predict the target genes of DE-miRNAs and to assess their involvement in stress- and pain-related pathways. The results of NGS showed DE-miRNAs between the treatments and across time points. Gene ontology demonstrated that these miRNAs are involved in stress- and pain- related pathways. Therefore, miRNAs have the potential to act as biomarkers of pain and stress in piglets during castration. These findings provide a molecular basis for objective pain assessment and support the development of evidence-based welfare strategies in pig production.

Effect of plumage condition on the welfare of end-of-lay broiler breeders exposed to a transport model

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End-of-lay broiler breeders are exported for slaughter from Denmark to other EU member states year-round, at approximately 60 weeks of age. The hens often have poor plumage condition due to repeated mounting of the hens by roosters, feather pecking, wear and tear from the environment, and nutritional factors. Poor plumage condition (i.e., reduced feather cover and more exposed skin) could pose an increased risk for injuries and thermal stress during transports occurring during colder seasons. Herein, we present an experimental model study of poultry transport to gain knowledge on whether transport at lower temperatures affects the welfare of broiler breeder hens and if these effects depend on plumage condition. The transport model we designed contained two replicates (nTotal = 144, 62-week-old broiler breeder hens, Ross 308) in a constrained Latin square design with each replicate acting as its own control while rotating through simulated transports at three different temperatures (5, 12 and 20 □C) representing a realistic temperature range of Danish outdoor conditions. Hens were scored for plumage condition using the methods of Bilcik and Keeling (1999) and designated as having Better (score ≥19), Moderate (16 – 18), or Worse (0 – 15) plumage condition. Hens were assigned to groups of 6 in commercial transport crates (N=12 crates per replicate, 50 x 85 x 30 cm, i.e., 708 cm^2 per bird) with two birds from each level of plumage condition in each crate to reflect possible mixing of birds during transport. The birds were exposed to the model conditions in a temperature-controlled room equipped with cooling capabilities and real time monitoring of temperature and humidity. In addition to monitoring room temperature, each crate was equipped internally with a temperature and humidity sensor. Furthermore, in the experimental room multiple thermal sensors were used to determine the room temperature. During each replicate, a random sample of three crates were removed successively after 3, 6, 9 and 12 h, respectively, of exposure to the conditions. Twelve hours was chosen as the maximum duration, corresponding to the maximum time that birds can be transported without provision of feed (Council Regulation (EC) No1/2005). The welfare of the birds was assessed before and after exposure to the simulated transport conditions using a variety of physiological and behavioural indicators. To monitor thermal stress, half of the birds (n = 72) were orally administered a temperature datalogger to approximate core body temperature which will be compared to cloacal temperature. Additionally, birds were weighed and an injury assessment (including the number and severity of scratches and wounds) was performed. Venous blood samples were taken from a subset of birds (n = 24 per simulated transport) to quantify glucose, lactate, electrolytes, haematocrit and venous blood gases. After each replicate, hens were placed into behavioural test pens to assess feed and water intake, thermoregulatory and comfort behaviour, and activity level. This newly established model of poultry transport will ultimately provide knowledge on the effect of plumage condition of end-of-lay broiler breeder hens transported for slaughter during colder seasons. The included animal-based measurements will provide information about possible welfare concerns that can be further studied.

Indicators for assessment of animal welfare on euthanized or deceased animals

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There are very few studies that assess animal welfare by examining carcasses at animal by-product rendering facilities. The existing studies consistently show that numerous animal welfare–relevant findings were identified in fallen stock and that these findings occur frequently. Based on this evidence, fallen stock were included in the NaTiMon project (National Animal Welfare Monitoring) as one important data source. The aim of the NaTiMon project was to design a national animal welfare monitoring system for Germany in order to generate knowledge about the current status of animal welfare in livestock husbandry. To achieve this goal, welfare indicators from the literature were selected, and a set of indicators for different livestock species was tested for their practical on-site applicability. Indicators for assessment on cattle and pig carcasses were selected that can be evaluated without dissection, relying solely on visual inspection and, where necessary, palpation. This approach allows a large number of carcasses to be examined within a short period of time without significantly disrupting the workflow of the plant. All indicators should be reliably measurable, provide a valid assessment of animal welfare, and it should be possible to train auditors without veterinary education to carry out the assessment. After a preliminary identification of indicators from the literature, the selection of indicators for testing was carried out through an expert panel. The resulting set of indicators was tested at ten facilities in eight federal states in Germany, with assessments conducted on one to two days per facility. On each day, between 20 and 100 animal carcasses were evaluated, resulting in a total of approximately 800 animal carcasses being examined. Data collection during ongoing operations was feasible in all facilities visited; however, carcasses had to be set aside for examination, which resulted in additional workload for the staff. Finally, a selection of six indicators was established, four of which apply to both cattle and pigs, and two specifically to pigs. In addition to the correct performance of emergency killing (indicator 1), the focus is on signs of prolonged illness prior to death, such as severe emaciation (indicator 2) and the presence of decubital lesions (indicator 3), as well as severe swellings (indicator 4) and, additionally in pigs, severe hernias (indicator 5) and the loss of tails and ears (indicator 6). At the end of the project, the indicator set, along with guidelines for data collection and instructions for conducting on site visits and ensuring biosecurity, was published. The results of the assessments were not statistically analyzed within the NaTiMon project, as the primary objective was to define a set of indicators and to test the feasibility of collecting these indicators. The project has shown that collecting data on fallen animals is possible and useful for maintaining the status quo of animal welfare in Germany. Regular monitoring of indicators can help identify acute animal welfare issues and inform the development of preventive measures for the future. This work was funded by the Federal Ministry of Food and Agriculture (BMEL) based on a decision of the Parliament of the Federal Republic of Germany, granted by the Federal Office for Agriculture and Food (BLE).

Field Feasibility and Repeatability of Standardised Protocols for Turkey Welfare Assessment

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Despite regulatory and private efforts to improve animal conditions from farm to slaughter, the practical application of welfare assessment protocols remains challenging, as existing systems still face limitations in comparability and reproducibility across European countries. As part of the EUP AH&W SOA9 activity, this study aims to harmonise turkey welfare assessment across Member States by reviewing existing protocols and developing standardised procedures for selected animal-based measurements (ABMs), evaluated on-farm and at slaughter. The validation of the proposed standard protocols focuses on field feasibility, inter-observer and intra-observer repeatability. In turkeys, breast blister (BB) and footpad dermatitis (FPD) were identified as key indicators. The comprehensive bibliographic review revealed substantial differences in scoring methods and evaluation conditions, highlighting the need for methodological harmonisation. Therefore, the following standardisation approach was proposed to fill the gap of knowledge. For feasibility, the selected ABMs have been visually assessed in multiple slaughterhouses in three different countries (France, Italy, and Spain) to ensure representativeness, and account for variability in animal and operational conditions. For repeatability, the field evaluations have been carried out by multiple observers per country who completed a harmonized training phase prior to data collection. During this phase, photographs and videos were jointly reviewed to align scoring criteria and to ensure consistent ABM interpretation. Field assessments started only after a predefined level of inter-observer agreement had been achieved. BB, defined as a form of contact dermatitis characterized by a pathological response of the skin in contact with hard surfaces, have been evaluated on the slaughter line after plucking using a binary scoring system (0 = absent; 1 = present), with a lesion threshold of ≥ 0.5 cm². FPD, described as a contact dermatitis of the plantar surface of birds' feet affecting both the skin and subjacent tissues, have been classified into the three scoring categories, based on lesion extent on the metatarsal footpad. For intra-observer repeatability, each left foot has been assessed after removal from the slaughter line, and independently evaluated three times by each observer. Preliminary results indicate that the standardised protocols are applicable under diverse field conditions, with inter- and intra-observer repeatability currently ongoing. The resulting dataset will be integrated into a European knowledge platform summarising feasibility, repeatability and implementation effort, thereby strengthening harmonised and comparable turkey welfare monitoring across Member States.

Animal welfare in pork production: Farmer and Veterinarian perspectives

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Good animal welfare is known to enhance productivity, contribute to improved economic margins, and long-term sustainability. However, clearer stakeholder understanding of these relationships is needed to support industry decision making for broader adoption of welfare strategies. The objective of this study was to examine how pork industry stakeholders perceive animal welfare and its role in business decisions by assessing: (1) how producers and swine veterinarians define animal welfare, including the metrics they use to monitor it on-farm; and (2) stakeholder understanding of how management procedures influence welfare, whether decisions are driven by welfare or productivity concerns, and whether barriers to implementation exist. Concept elicitation interviews with open-ended questions were conducted to capture stakeholder experiences with on-farm welfare management. Ten participants were interviewed; four experienced swine managers and six veterinarians, including eight from Canada and two from the United States. Interviews elicited detailed descriptions of participants' definitions of animal welfare, the management practices they implemented and the motivations behind management decisions, welfare and productivity indicators used, perceived impacts of management practices on staff well-being, and views on the interconnections between welfare and production performance. Generally, producers did not provide a clear definition of animal welfare, but referenced welfare indicators they use to assess welfare; these included primarily visible, physical measures related to economically relevant performance metrics (i.e., skin injuries, disease), rather than indicators reflective of affective states (i.e., behavioural indicators). Respondents acknowledged the value of good welfare for profitability and staff well-being, but welfare considerations were secondary to economic priorities, and practices involving upfront costs were rarely adopted unless mandated. This study indicates that, while animal welfare is broadly acknowledged as a key component of pork production, it is not yet fully understood or integrated into the workplace culture or business models of pork production. This may reflect a limited appreciation of the broader long-term benefits of welfare for economic viability. Future research that integrates welfare improvement strategies and practical assessment tools with economic analyses could strengthen understanding of its contribution to long-term business viability.

Harmonization and Standardization of Indicators for Assessing Sheep Welfare

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Within the European Partnership for Animal Health and Welfare (EUPAHW), a group of experts from Research Performing Organisations in five EU countries (France, Spain, Estonia, Ireland and Italy) carried out a literature review for the harmonisation of selected animal-based welfare indicators for sheep. The activity was conducted under Task 2, coordinated by ANSES and UAB and contributes to Set of Activities 9 (SoA9), aimed at developing a European Knowledge Platform on animal welfare. A pre-existing digital database (More Welfare project OC/EFSA/ED/2022/02), including peer-reviewed studies previously screened for relevant welfare indicators, was used as the main starting reference. The objective was to harmonise and standardise existing assessment indicators through literature review and systematic data extraction. A comparative analysis of peer-reviewed studies and documented methodologies from the database identified methodological similarities and differences and supported evidence-based harmonised approaches based on validated protocols.The indicators assessed were: panting, nasal discharge, fleece cleanliness, tail length, social withdrawal, qualitative behaviour assessment (QBA), human approach test, and lameness and leg injuries. Data were extracted into a structured Excel dataset including sample size, production stage, farming system, assessment location (farm or slaughterhouse), study design, protocol characteristics, body regions assessed, scoring methods, welfare criteria and thresholds. This enabled comparison of methodological heterogeneity and identification of strengths and limitations. Following comparative analysis, methodologies for indicator harmonisation were proposed. These proposals were reviewed by sheep welfare experts to assess scientific robustness, feasibility and applicability across production systems.For most indicators, widely used protocols were confirmed as valid and requiring no substantial modifications. The only exception was panting, for which a harmonised simplification was proposed: the original 7-point scale (Lees et al., 2019) was reduced to a 5-point scale to improve clarity, inter-observer agreement and on-farm feasibility without compromising sensitivity. Next steps include a bibliographic review to evaluate feasibility and repeatability of each method. Inter- and intra-observer agreement tests will quantify reliability. Field validation of the harmonised panting protocol will be conducted on at least three farms per country representing different systems, with assessments performed by at least two observers and repeated three times to ensure robustness.This coordinated effort enhances consistency, comparability and implementation of sheep welfare assessment across countries, supporting harmonised methodologies within SoA9 and strengthening the scientific basis for future monitoring frameworks. This work was co-funded by the European Union's Horizon Europe Project 101136346 EUPAHW.

Sensor-based monitoring of behavioural adaptation of dairy calves during regrouping from individual or social housing to group housing

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Regrouping of dairy calves from individual or pair housing to larger group pens represents a major management transition and may induce behavioural and physiological stress responses. Automated monitoring approaches can support objective and continuous welfare assessment under practical conditions. The aim of this study was therefore to investigate behavioural adaptation of dairy calves during regrouping using accelerometer-based monitoring. The study was conducted on a commercial research farm and included 61 Holstein calves housed either individually (n = 31) or in pair/small group housing (n = 29) during the first two weeks of life. At an average age of approximately 14 days calves were regrouped into larger group pens equipped with an automatic milk feeding system. Behaviour was continuously recorded using tri-axial accelerometers (HOBO Pendant G) attached to the hind leg of each calf. Sensors recorded acceleration and position data at 10-second intervals for approximately 2.5 days before and 2.5 days after regrouping. A previously developed and video-validated classification algorithm was used to assign behavioural states (lying, standing, activity) from the raw sensor data (overall accuracy ≈ 88 %, Cohen's $\kappa \approx 0.75$). Behavioural time budgets and bout characteristics were calculated for each calf and analysed using linear mixed models including period (before vs. after regrouping), initial housing (individual vs. social), and their interaction. Across the observation period calves spent most of their time lying, followed by standing and activity. Regrouping was associated with clear behavioural changes in both housing groups. Standing time increased after regrouping, suggesting increased exploration and social interaction in the new environment. Calves previously housed individually showed increases in both standing and activity after regrouping, whereas calves with prior social housing mainly showed increased standing time but tended to reduce lying and activity durations. In addition to changes in time budgets, behavioural bout structure also changed. In individually housed calves, lying and standing bouts became longer after regrouping, indicating reduced fragmentation of these behaviours. Circadian patterns of behaviour remained largely stable across the observation period. The results demonstrate that regrouping represents a relevant adaptation phase for dairy calves and that prior social experience influences behavioural responses to this management change. The study further highlights the potential of accelerometer-based monitoring systems to provide objective and continuous welfare indicators in calf rearing systems

On-farm self-assessment – tools to improve cattle welfare

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Since February 2014, in addition to daily routine inspections, livestock farmers in Germany are obliged to check the welfare status of their animals within the framework of on-farm self-assessment (Animal Welfare Act § 11(8)). For this purpose, they have to use animal-based indicators (termed "Animal Welfare Indicators"). This should ensure that the requirements of § 2 Animal Welfare Act are met. The aim of the project "Eigenkontrolle Tiergerechtheit (EiKoTiGer)" was to test the feasibility of the guideline for on-farm self-assessment (dairy cows, calves and beef cattle), published in 2016, and to develop an online training course, tools for data recording as well as reference values, which help to appraise the results. The guideline was developed by a group of cattle experts. In a first step, indicators suitable to detect relevant animal welfare issues, appropriate for on-farm self-assessment, were identified and described. The descriptions cover background information and definitions including result formulas and illustrations. Within the "EiKoTiGer" project, the feasibility and reliability of these indicators were investigated on 44 cattle farms. Based on the results and farmers' feedback the guidelines were revised and data recording tools were further developed. In addition, an online training course was set up to support reliable assessment of the indicators. Reference values for evaluating the farms' own results were elaborated in a multi-stage process by means of a Delphi survey, literature review and expert panels involving different stakeholder groups. The guideline for dairy cows, calves and beef cattle (updated edition published in 2020) supports cattle farmers to carry out self-responsible and systematic inspections on the welfare status of their herds (https://www.ktbl.de/themen/tierwohlbewertung#c2729). The 44 farmers involved in the pilot study rated the guideline as largely self-explanatory. The online training course (https://tierschutzindikatoren-schulung.ktbl.de) enables participants to apply the indicators reliably and in a standardised way under farming conditions. The course includes exercises with a feedback function and an online test. Obtaining a certificate is possible with a positive test result. To support digital data recording, an Excel® based tool was developed (https://www.ktbl.de/webanwendungen/tierschutzindikatoren-erhebung/). The tool includes automated data processing and provides a summary of the results at farm level. In addition, printable sheets are available for a paper-based data recording in the stable. Furthermore, farmers can compare their results with reference values and thereby determine, whether action to improve animal welfare on their farm is needed (https://www.ktbl.de/themen/tierwohlbewertung#c4100). The German Animal Welfare Act requires livestock farmers to assess and evaluate animal-based indicators in order to monitor whether housing conditions meet their animals' need. The guideline and the tools presented are intended to support cattle farmers to carry out systematic assessments of animal welfare in their herds and are freely available at: http://www.ktbl.de/themen/tierwohlbewertung. They help to identify weaknesses and to improve cattle welfare and support herd management based on the systematic assessment of reliable objective animal welfare indicators.

Promoting Cattle Welfare Planning

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Animal welfare in cattle farming is of high ethical relevance and closely linked to resilient livestock systems, resource efficiency and GHG emissions. Despite the duty to on-farm self-assessment since February 2014 (Animal Welfare Act § 11(8)), there is still considerable room for improvement in animal welfare in cattle farming. The predominant welfare challenges involve multifactorial production diseases such as lameness, udder health disorders and metabolic imbalances. These conditions can be effectively addressed only through preventive, risk-based management approaches. The concept of animal health and welfare planning follows a cyclical and continuous process encompassing systematic monitoring, definition of target values, risk analysis, implementation of measures and regular evaluation. This structured management concept has been extensively investigated and successfully implemented in various on-farm projects, yet its adoption in German cattle farming remains limited. Against this background, the project 'InnoRind' aimed to facilitate the practical implementation of animal welfare planning by developing a practice-oriented guideline and an accompanying online platform. These tools are designed to support preventive herd management and to contribute to improved animal welfare and sustainability in cattle farming. In the project 'InnoRind', the concept of animal welfare planning was further developed based on existing scientific knowledge and adapted to the needs of the target group. The approach was implemented through a practical guide and the digital platform https://www.tierwohlplanung.de/, where existing tools, supplementary information and materials on animal welfare assessment were systematically structured and made accessible. Successful implementation of animal welfare planning requires active responsibility and engagement by animal caretakers. Accordingly, the primary target group for communicating the concept comprised German speaking cattle farmers in practice as well as in education and training contexts, followed by advisors and trainers. For this purpose, a clearly structured and easily understandable animal welfare planning scheme was developed, illustrating the cyclical and continuous process of implementation: (1) monitoring and evaluation of animal welfare ('See'), (2) risk analysis ('Understand'), and (3) planning and implementation of measures ('Improve'). Complementing the guide, the website https://www.tierwohlplanung.de/ presents the concept concisely, provides practical implementation tips, and offers access to existing tools for welfare planning in dairy cows, calves, and beef cattle. Each tool is described with its potential applications and includes direct links to the respective providers. Since the introduction of mandatory self-monitoring of animal welfare (§11(8) Animal Welfare Act, 2014), a variety of scientifically validated tools for monitoring cattle welfare have been developed in Germany. Embedding these tools within a structured, preventive management framework enables systematic use of monitoring data for herd-level decision-making. Guideline (https://www.tierwohlplanung.de/ratgeber), digital platform (https://www.tierwohlplanung.de/), and tools (ibidem) were developed to enable practical implementation of the animal welfare planning concept by communicating its method and benefits and providing easy access to relevant tools. This approach can increase farmer engagement, support preventive herd health management, and help to reduce disease-related losses and medication use. Consequently, it fosters sustainable improvements in animal welfare and can enhance resilient livestock systems and resource efficiency in cattle farming.

From Knowledge to Practice: Ensuring Long-Term Impact in the European Equine Sector through EUnetHorse and TANet

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The European equine sector remains structurally fragmented and insufficiently integrated into national Agricultural Knowledge and Innovation Systems (AKIS), limiting its capacity to respond to environmental, socio-economic and political challenges. Strengthening resilience requires coordinated knowledge exchange and long-term dissemination strategies tailored to the sector's needs. EUnetHorse addresses this gap by establishing an active multi-actor network across nine European countries (France, Portugal, Spain, Belgium, Germany, Romania, Poland, Finland and Switzerland). The project enhances the resilience and performance of equine farms through three dimensions: (1) socio-economic performance and crisis preparedness, (2) animal welfare and health, and (3) environmental sustainability. A central innovation is the structuring of National Horse AKIS networks, ensuring balanced knowledge flows while accounting for regional diversity. Grassroots solutions are identified, technically assessed and translated into accessible practice-oriented formats. Breeders, advisors, trainers, researchers, policy makers and sector representatives engage in workshops, demonstration events, cross-visits and targeted training, fostering peer learning, co-creation and improved adoption of solutions. The Communication and Dissemination (C&D) strategy is conceived as a sustainability instrument rather than a purely promotional tool. Project outputs are integrated into the EUFarmBook platform, the EUnetHorse website—maintained for at least two years beyond project completion—and the Zenodo repository, ensuring open access and long-term availability of results. A key pillar for durable uptake is the Trainers and Advisors Network (TANet), launched in April 2024 and currently comprising 70 members from nine countries. TANet members identify advisory needs and co-develop training materials, embedding project results into national advisory systems. The network is expanding beyond partner countries and is structured to operate autonomously beyond the project lifetime. Target indicators include 20 members in the international TANet, 80 trainers and advisors within national TANets, and outreach to 300 trainers and 120 advisors across participating countries. National Facilitators act as knowledge brokers, aligning activities with existing training and advisory structures. By the end of the project, a digital training kit compiling methodologies, technical sheets, posters, videos and other practice-oriented materials will be developed. To reinforce impact, a strategic C&D plan ensures coherent EU-level branding while allowing national adaptation. Dissemination combines mass communication and targeted channels, including 80 EIP-AGRI practice abstracts, biannual newsletters and two policy briefs. Materials are translated into eight partner languages to maximise accessibility. Since project initiation, social media engagement has increased by more than 300%, reflecting growing sector involvement. By integrating structured AKIS networks, participatory co-creation and a sustainability-oriented communication model, EUnetHorse proposes a replicable framework for durable knowledge transfer in the equine sector, strengthening farm resilience beyond the lifespan of EU-funded projects.

Improving resilience of equine farms: EUnetHorse's methodology for assessment of solutions and best practices in the areas of animal welfare, socio-economics and environmental sustainability

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EUnetHorse is a collaborative EU project aiming to collect, process and disseminate evidence-based knowledge and best practice examples among equine farmers in the thematic areas animal welfare, socio-economics, and environmental sustainability. The ultimate aim of the project is to increase resilience of equine farms. Here, we provide information on the methodology used for the selection and evaluation of solutions. The process includes three steps: (1) data collection, (2) assessment, and (3) feedback from end-users. The objective of this process is to select solutions that have the most pronounced effects on farm resilience. (1) To collect information on farmers' needs and existing solutions, 365 farmers across the 9 participating countries were surveyed. Additionally, operational groups, focus-groups and national projects were consulted, and literature searches were conducted. This resulted in 72 prioritized needs and more than1000 identified solutions. Based on criteria such as readiness level for implementation, scientific validity, availability, and format (digital or not), a minimum of 120 solutions are selected for assessment, with a target of 80 positively assessed, adapted, and ready-to-implement solutions. (2) Using a multi-actor approach involving various stakeholders and experts such as farmers, scientists, advisors, and trainers, an assessment grid was used during national workshops, to evaluate: a) performance, based on the solution's impact on 15 areas distributed across the three thematic areas (e.g., environmental performance: climate change, biodiversity, water and land management), and scored on a scale from -2 (negative) to +2 (positive); b) resilience, based on how the solution mitigates six predefined external challenges such as events of inflation or disease outbreak; and c) the cost-benefits-balance. Scores are calculated for each challenge and aggregated into an overall resilience score. (3) Using a cost-benefit analysis, a scenario with the adopted solution is compared to a scenario without such solution in place. Evaluators identify stakeholders who may be affected by the solution and catalogue the associated benefits and costs across the three thematic areas. Subsequently, solutions are presented at European workshops to discuss scores and cost-benefit analyses, filtering out those with negative scores. For successful solutions technical-sheets and posters are created. At this stage, to facilitate understanding of the solutions' overall impact within a given topic, total performance and resilience scores are also rescaled to the -2 to +2 scale. End-user acceptance is tested during demonstration days and through online surveys. Technical sheets and posters will be made freely available on the EUfarmbook website (eufarmbook.eu), providing a public resource for all stakeholders. The overall methodology itself is also reviewed with the project's trainers- and advisors network, and national stakeholders to ensure clarity and usability across partner countries. This participatory and scalable approach enables EUnetHorse to quantify the performance and resilience contributions of hundreds of solutions, ensuring that the final portfolio is robust, cost-effective, and widely acceptable to farmers, trainers, and advisors.

EUnetHorse : Recognising the equine sector : unlocking its contribution to Europe's climate, rural and competitiveness goals

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EUNetHorse is the first European agricultural project dedicated to equine animals and is setting up a new dynamic across the European equine sector, by fostering connections among the different equine sectors, geographical levels and categories of actors. The overall objective of EUNetHorse is to establish an active multi-stakeholder network in order to increase the resilience and performance of equine farms. First, following a bottom-up approach, 365 farmer interviews and 9 national workshops were conducted across the nine countries in order to identify and prioritize farmers' needs and existing solutions. Then, at the EU level, 72 needs were prioritized and organised into 9 priority areas (profitability, working conditions, accommodation and equine welfare, biosecurity and emerging diseases, adaptation to climate change, biodiversity, grassland management, land access, training and knowledge transfer). More than 1,000 solutions and best practices were identified and characterised (readiness level (redefined scale), digital or not, farm or industrial level, availability in Europe). A total of 428 solutions met the minimum requirements. A matchmaking process between needs and solutions was performed at the EU level and some solutions were grouped allowing to identify and analyse gaps (i.e. no relevant solution for a given need). 17 gaps were identified at the industrial level and 5 at the farm level. A total of 41 needs were identified as requiring significant research and development support. As a conclusion of this report, a policy brief was produced and will be disseminated soon in order to obtain strategic EU-level support to unlock the full potential of the equine sector which is at a crossroads facing external challenges. The Policy brief is structured into 4 parts: 1) an introduction highlighting the importance of the equine sector from a socio-economic point of view; 2) a focus on the sector's key contributions to European objectives: an attractive agricultural sector for young people and particularly women, maintenance of permanent grasslands, high value chain, preservation of fragile ecosystems (Camargue, Konik Polski), a sector that uses few pesticides, non-relocatable and inclusive jobs that contribute to rural development (tourism, services, culture); 3) urgent policy actions (reaffirming the agricultural status of equines and reintroducing equines into agricultural statistics); 4)the need for R&D support (ensuring the eligibility of equine animals and activities in FP10 calls and focusing on more specific themes such as adapting existing digital tools at the European level). The next EU Framework Programme for Research and Innovation (FP10, 2028–2034) is currently under negotiation. Supporting the specific needs of the equine sector within this future programme represents a key opportunity to direct funding towards the knowledge gaps identified by EUnetHorse. It is therefore important that the scientific community is informed of these priorities in order to position itself, if necessary, to respond to EU calls for proposals.

Group discussion Part 1

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This project session is dedicated to the EUnetHorse project and its future perspectives. The three previous presentations introduced the project and its main results. EUnetHorse is the first European agricultural project dedicated to equine animals. Its overall objective is to establish an active multi-stakeholder network in order to increase the resilience and performance of equine farms. In this perspective, the project has created a new dynamic across the European equine sector by fostering connections among the different equine sectors, geographical levels, and categories of stakeholders. Its aim is to collect and disseminate research results in order to make them useful and accessible to the equine industry. Another important outcome of the project has been the identification of major research gaps within the equine sector.Building on these results, the second part of this session will be dedicated to discussion and collective reflection with the objective of creating consortia for future European projects dedicated to equines. This working session will therefore be divided into two parts: Part 1 of the group discussion:Before the coffee break, we will summarize the main research gaps identified through the EUnetHorse project and present relevant European calls for proposals that will have been previously identified. These gaps and calls will be discussed together in order to identify funding opportunities. Additionally, participants will be invited to express their interest in potential future projects.

Group discussion Part 2

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Following the first part of the group discussion, here is the programme for the second part:After the coffee break, we will organize several parallel workshops dedicated to project development. Each workshop will be chaired by a facilitator. The 45-minute session will be dedicated to initiating reflections on the project content and on potential partnerships between participants and other researchers, institutions, and professionals who may be identified during the discussions. By the end of these workshops, we aim to have initiated collaborations and consortia and to have begun preparing future European project proposals, including possible timelines, deadlines, and project contents.Finally, the objective of this session is to stimulate the emergence of new research projects for the equine sector that address real and pre-identified needs, correspond to relevant European funding calls, and are supported by new research consortia willing to collaborate on these topics.

Thematic expert panel insights on state-of-the-art digital tools and technologies in the livestock sector

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The aim of this study was two-fold: (i) to identify challenges and opportunities related to state-of-the-art digital tools and technologies in the livestock sector and (ii) to propose solutions that could help increase efficiency and adoption rates based on insights from expert stakeholders. A thematic expert panel (TEP; n=10) of software developers (n=2), technology providers (n=4), and stakeholders (farmers, consultants, breeding companies) that use digital solutions (n=4) was created within the framework of Digi4Live project (https://digi4live.eu/); their activities, geographical distribution, and diversity of species of interest were considered. Five TEP meetings were scheduled to discuss: (i) recent developments in digital tools and technologies, (ii) user requirements, (iii) level of automation and potential for customization, (iv) market penetration, and (v) associated costs. Insights from the first two TEP meetings indicated that artificial intelligence (AI), internet of things (IoT), global positioning systems (GPS) and satellite devices, robotics, and sensors for monitoring environmental conditions and animal performance are key recent advancements. Poultry, swine, and dairy cow sectors were identified as the main adopters because they tend to have higher profit margins and producers with higher levels of education. Most experts (63%) considered that available technologies are moderately user-friendly for farmers. Major user requirements included account creation (100%), internet access (86%), documentation (86%), and data input (63%). Limitations regarding real-time data monitoring, interpretation and integration, poor connectivity, complexity, financial barriers, and data ownership issues were reported as the most frequently cited user complaints that hinder adoption. Improving available products based on user feedback providing both online and hands-on training, and AI-assisted decision support tools were considered imperative to increase adoption and support farmers to use digital technologies effectively. Emerging trends expected to shape the future of user requirements included new legislations, data sharing, predictive animal health status, animal identification and herd level metrics, precision feeding and resource use efficiency. Future TEP meetings are expected to provide further valuable insights regarding challenges, opportunities, and solutions for adoption of digital technologies in the livestock sector. The work is part of the Digi4Live project (ID: 101134866), funded by the European Union.

UHF RFID technology in livestock applications: synthesis of French case studies in the ruminant sector over 10 years

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Widely adopted in dairy farming, wearable technologies have struggled to scale to small ruminants and extensive systems due to prohibitive costs at whole-herd level. Meanwhile, mandatory RFID (Radio-Frequency Identification) ear tags in parts of Europe remain underutilised because operational constraints limit their practical value. Could UHF (Ultra High Frequency) ear tags overcome these barriers and provide an affordable, frugal, and scalable solution across multiple applications? This presentation illustrates several use cases of UHF ear tags and discusses the remaining technological and systemic challenges to large-scale adoption. Compared with low-frequency RFID, UHF RFID offers longer reading ranges without restraining animals, the ability to process high-density tag populations efficiently, and low per-animal costs thanks to passive (battery-free) tags, key assets for scalable and frugal precision livestock farming innovation. Over the past 10 years, IDELE conducted a series of trials and projects (H2020 TechCare, Salto, Horizon Europe Open Agri, Pacapit, OviFlash, Aboire) to assess the practical potential of UHF RFID in ruminant farming. Use cases have covered: (i) traceability with batch readings using RFID gates; (ii) herd management through rapid counting and detection of missing individuals (inventories); and (iii) monitoring of "points of interest" (e.g., passages, feeders, water points) to explore long-term behaviours and derive potential health-related indicators. These investigations were performed in both indoor and outdoor conditions, including settings without grid electricity, using solar stations – highlighting pathways to reduce infrastructure constraints, energy needs, and operational complexity. This work also supported the co-development with industry and research into pragmatic and innovative solutions: a transportable UHF RFID reading device combining fully embedded hardware/software, a cloud database with a user interface, a mobile application offering the possibility of offline and low-energy use, innovative antenna enabling indoor location. This summary synthesizes the main technical lessons learned across this exploratory program (based on laboratory's measurements and real-farm deployments). This presentation highlights the co-evolution of tools and use-case needs, showing how technology has progressed from early batch management and individual sheep traceability to a range of on-farm precision livestock farming solutions for both cattle and small ruminants. Beyond performance, each trial provided actionable insights into the technology's advantages, limitations and the precautions required for reliable on-farm use with the objective of guiding robust, user-friendly, and cost-effective deployment of UHF RFID-based tools in livestock systems. UHF RFID widely adopted in logistics and retail, is now being standardized by ISO for animal identification and will soon provide an additional option for electronic livestock identification.

Machine Learning models for Auto-Annotation of Behavioral Data to Generate Digital Ethograms

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The application of machine learning (ML) models to ethology promises to transform the study of animal behavior and provide new insight into animal health, production, and welfare. However, the development of these models is increasingly constrained by the time-intensive and subjective nature of manual coding or annotation of behavioral data. The use of wearable Inertial Measurement Units (IMUs) that provide continuous, animal-level behavioral data streams offers an opportunity for more objective behavior classification and validated behavioral metrics. The IMUs also generate high-frequency, long-duration datasets, and thus converting raw IMU signals into structured, high-quality behavioral labels remains a critical bottleneck for scalable ethologically based ML models. This work presents an ML framework first for annotating IMU data for key behavioral states and events (e.g., standing, lying, left- and right-side lying, feeding) in sows. Frame-by-frame analysis of video data can be used when needed as a high-resolution reference modality during annotation. This enables precise temporal alignment of IMU output with behavior and unparalleled delineation of ethological event transitions and their durations. This high-quality data set provides the foundation for the second aspect of our novel ML framework, the auto-annotation of time-series IMU data using ML models. A total of 184 hours of synchronized IMU outputs (6,593,272 data points) and video were recorded and annotated. The models are trained on 138 hours and validated on 46 hours of this data. Over 1,634,000 data points were subjected to the trained Random Forest Classifier model that predicted sow posture with greater than 90% accuracy, specificity, and sensitivity. The resulting auto-annotation ML pipeline substantially reduces manual coding time and produces standardized, reproducible behavioral labels from continuous IMU data. Adopting a digital ethogram and auto-annotation workflow accelerates the creation of foundational behavioral datasets, supporting downstream deep-learning applications, individual-animal modeling, and cross-study comparability. More broadly, this work demonstrates how time-resolved ML approaches can bridge the gap between primary IMU sensor outputs and biologically meaningful ethological representations in Precision Livestock Farming.

Individual fattening pig growth profile estimation

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Monitoring fattening pigs is important to ensure adequate production and welfare during the rearing period. Pig growth is a rather stable variable which is directly linked with production but also indirectly with a range of health issues. Therefore, monitoring and modelling pig growth can provide important information, which can be used for precision feeding or digital twins. However, up until now, the body weight and growth of fattening pigs is often monitored on the group level, thereby concealing variation between individual pigs or issues on the individual pig level. This study explores using the existing mechanistic InraPorc model to estimate individual fattening pig growth profiles, integrating automated body weight and feed intake data. Data of two pig batches was used (1) 120 pigs, aged 68–167 days with a body weight between 25.2–106.8 kg and (2) 120 pigs, aged 67–179 days and 23.5–121.5 kg. The pigs were housed in 8 pens within a single compartment, where feed intake measurements were available in half of the pens via feeding stations. Daily body weight measurements of all individual pigs were available in all pens through weighing scales in front of the feeding stations or drinkers. The InraPorc software was used to calculate the average pig profile of batch 1, which consisted of the 4 main model parameters. This average pig profile and an initial individual pig body weight acted as a baseline estimation for this study. Different scenarios were explored to update the optimal values for the 4 main model parameters for individual pigs on a daily basis. The scenarios differed in (1) the amount of data used (last step update or rolling update), (2) the initial guess for the model parameters (average profile parameters or last known parameters), (3) the use of feed intake data (with or without feed intake data), and (4) the batch of pigs tested (batch 1 or batch 2). The mean absolute percentage error (MAPE) was calculated for each pig to evaluate the performance of the individual profile estimation. All scenarios except one resulted in a numerical decrease of the MAPE compared to the average profile, although the decrease of the MAPE was not always significant. Last step updates resulted in the most flexible estimation of the pig profile, quickly adapting to changes in the animal profile, while the rolling parameter update scenarios resulted in more stable animal profile estimations reacting slower to changes in the animal profile, resulting in MAPE of 2.2-5.2 and 5.4-7.8% respectively. Using the last known parameters as initial guess presented a high risk for the optimization step to fail due to the model's imposed maximum growth, which created regions where the optimization function plateaued. Because the model in pens without individual feed intake measurements used the average profile's feed intake parameters, calibrated on the overall performance of batch 1, the individual profile estimation in those pens did not differ substantially from the pens with feed intake, with MAPE of 1.4-5.9 and 2.2-7.8% respectively. Similarly, the performance of the individual profile estimation in batch 2 did not differ substantially from the batch 1 profile estimation, with MAPE of 1.4-7.8 and 1.6-6.3% for batch 1 and batch 2 respectively, which was expected given the similarities between the batches. In conclusion, the results indicate that it is possible to estimate the individual fattening growth profile, although the error on the estimation differs depending on the method used to estimate the profile, offering new possibilities in daily pig body weight monitoring.

Risk Factors for Piglet Preweaning Mortality in Spanish Breeding Farms. Preliminary Results from the Bdporc-Smartporc Project

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BDporc (www.bdporc.irta.es/en) is a reference database of sow breeding performance in Spain and a strategic digital tool in smart livestock systems, providing standardized analytics that enable affiliated producers to identify strengths and weaknesses in their farm management. BDporc has recently launched the SMARTporc project to develop a data-driven digital decision-support tool aimed at optimizing sow longevity and piglet survival in breeding farms. The project focuses on 1) creating a Data Lake compatible with BDporc to integrate on-farm precision data with information about farm emissions, management, and pen characteristics, already collected in official databases, 2) identifying key factors affecting piglet survivability and sow longevity, and 3) developing a digital twin of pig a breeding farm to simulate management actions and evaluate their effects on piglet survival, sow longevity, and farm profitability. Preliminary results using BDporc data analyzed 10.5 M reproductive cycle records from 2.6 M sows in 630 commercial breeding farms (2020-2024). Pairwise correlation analyses identified that litter size and piglet losses traits are associated as the number of piglets born alive (PBA) was positively correlated with piglet mortality during lactation (r=0.28). Furthermore, Bayesian regression models fitted separately per cycle showed that higher piglet pre-weaning mortality was related with higher number of PBA, longer lactation lengths, and piglets weaned in the preceding cycle (i.e., highest posterior density interval at 95 % of 0.59-0.69, 0.42-0.52 and 0.24-0.34 in cycle 3, respectively). Moreover, percentage of piglet losses in the previous lactation impacted piglet mortality and this effect peaked at cycle four followed by a gradual decline thereafter. Season and year recorded during insemination were also associated with piglet mortality, with higher risks observed during spring and summer, as well as increased losses during the 2021–2023 period. Altogether, these initial results suggest that indicators of sow past performance can provide new insights to improve piglet pre-weaning survival.

Drone-based mapping of geese range use under heat across pasture systems: implications for silvopastoral agroforestry design

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Outdoor access can improve animal welfare in commercially kept geese (fattening and laying flocks). However, during hot conditions these benefits depend on pasture systems providing effective shade and shelter. Orthomosaics derived from unmanned aerial vehicles (UAV), combined with Geographic Information Systems (GIS), offer a scalable approach to analyse clustering and behaviour across pasture systems and structural features. Such information can support climate-adaptation strategies such as (agro-)silvopastoral systems. We aimed to implement a UAV workflow to quantify welfare-relevant space-use metrics of free-range geese across varying weather conditions and pasture systems. Between 30 July and 16 October 2025, six flocks of commercially kept geese (fattening and laying), ranging from approximately 300 to 2,200 animals per flock, were surveyed across contrasting pasture systems. These included maize-based systems (maize-only; maize-grassland) and grass-based systems with limited tree cover (grassland-only; grassland with young orchard or walnut trees and/or shade near buildings) as well as grassland with older fruit trees. UAV grid flights (typically 80-90 m above ground level; adjusted to avoid disturbing the flock) were used to generate orthomosaics, followed by spatial analyses in a GIS environment. Across six field days, 43 flights were conducted at different times of day and under varying weather conditions. Goose positions and clusters were extracted from orthomosaics using manual annotation and currently tested computer-vision approaches. Detection performance was validated across ground covers; initial tests in maize were promising due to high contrast between white geese and vegetation, but occlusion and crop-row structure can affect detectability. Response variables include cluster size, distance to structural elements, and shade-use proportion, modelled as functions of pasture system and environmental covariates using mixed-effects models. Weather data were obtained from the nearest German Weather Service (DWD) station. Pilot observations during hot conditions (air temperature up to ~34 °C) indicated strong thermal constraints on range use across all six flocks. Geese largely avoided ranging far into paddocks during the hottest hours. Two laying-goose groups on grassland with only small-canopy orchard or walnut trees lacked effective shade and clustered in barn-adjacent shade throughout the day. Three fattening-goose groups with access to maize also remained mainly indoors or close to the barn, and use of the maize range was detected only later in the evening. In contrast, a fattening-goose group on grassland with older fruit trees intensively used the limited tree shade, suggesting that not all shade types are functionally equivalent. Overall, these pilot data indicate that heat can strongly suppress range use in commercial geese and that shade effectiveness depends on shade type. Drone-based orthomosaics provide a practical on-farm approach to quantify these welfare-relevant space-use patterns and to support the design and evaluation of silvopastoral pasture systems for heat mitigation.

An introduction to bioacoustics with a focus on chicken vocal responses to birds of prey

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Bioacoustics is the scientific field that investigates sound production and perception in animals. Animal vocalisations can be used to extract information about the sender, such as physical characteristics or emotional, reproductive and health state. Such information is obtained by analysing the different types of vocalisations within a species and their temporal, frequency and spectral characteristics. This allows researchers to address various questions about animal behaviour, including individual differences in calling behaviour, changes in call structure under stress or disease and which call types are produced in specific contexts. Due to its wide range of applications, bioacoustics are increasingly applied in livestock farming, for example to detect potential health risks or stress in animals. Free-range farming of laying hens is becoming increasingly popular due to its benefits for animal welfare. However, outdoor access also exposes chickens to a higher risk of predation, creating a major challenge for farmers in preventing attacks by birds of prey. Therefore, this study aims to identify and describe the vocal responses of chickens in the presence of birds of prey, with the long-term goal of using these calls as the foundation for developing a potential deterrent system aimed at scaring off birds of prey. Audio and video recordings were conducted over three months on a flock of 990 hens and 20 roosters housed in a mobile housing system with daily access to a free-range area. Cameras and audio recorders operated daily from 9 am to 7 pm and captured the behaviour and vocalisations of the chickens within a maximum distance of 11.3 m from the mobile house. All recordings were screened for the presence of birds of prey, identified either visually or through their vocalisations. For each event, the corresponding video sequence was analysed to determine when chickens first perceived the birds of prey, defined as the moment a chicken (first animal) raised its head, elongated its posture and showed a behavioural change. Anti-predator behaviour was considered to end when the first animal and 75% of visible animals resumed normal movement. Audio segments between perception and the end of anti-predator behaviour were visually inspected and acoustically characterised to identify the call types produced. Our results show that chickens produce a variety of call types in response to the presence of birds of prey. Calls produced by roosters differed from those produced by hens. Chickens often emitted call types not specific to alarm situations during the presence of birds of prey. However, roosters frequently produced aerial alarm calls, suggesting that they may be suitable candidates for developing a vocalisation-activated deterrent system. However, as group composition may influence calling behaviour, further recordings in flocks with different social structures are planned to evaluate whether aerial alarm calls are consistently produced across the group compositions commonly found in chicken husbandry. This research is funded by the Lower Saxony Ministry of Science and Culture and the Volkswagen Foundation as part of the zukunft.niedersachsen program within the ZERN project.

Eyes & Ears – the auditory perspective: evaluating the potential of sound-based monitoring for livestock health, behaviour and welfare

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Bioacoustic approaches have gained increasing interest for monitoring health, behaviour and welfare in farm animals remotely, continuously and non-invasively. Compared with other sensor technologies, acoustic systems require relatively low computational power and data storage, facilitating practical on-farm deployment. However, important challenges remain before bioacoustics can be reliably implemented under commercial settings. Technical limitations related to complex and noisy farm environments continue to hinder the translation of experimental methods into robust tools, while the sound outputs of livestock species remain incompletely understood, making it unclear which health or welfare conditions can be consistently detected using sound. The newly initiated Eyes & Ears project, part of the European Partnership on Animal Health and Welfare and co-funded by the European Union's Horizon Europe Project 101136346 EUPAHW, aims to facilitate the identification, development and validation of on-farm surveillance tools based on both visual and auditory monitoring. Within this framework, the task dedicated to auditory monitoring brings together 12 partners from seven European countries to evaluate the potential of bioacoustics across multiple species, including pigs, poultry, cattle and small ruminants, and across different production stages and husbandry systems. The task will generate case studies, performed in experimental facilities and commercial farms, exploring the use of sound to monitor, for example, respiratory disease, resource use, maladaptive behaviours, and signs of both negative and positive affective states, while examining technical feasibility and methodological considerations for on-farm application. A key objective is to identify which health and welfare indicators show potential for feasible monitoring using audio alone, and which may require multimodal approaches combining sound with other sensing technologies. In parallel, the project aims to promote collaboration across research groups by encouraging standardized methodologies, cross-partner studies, and the development of best-practice guidelines. Running over three years (2026-2028), this work will contribute to establishing a stronger scientific and methodological foundation for livestock bioacoustics and support the transition of sound-based monitoring from experimental research towards practical on-farm applications to assist farmers and improve animal welfare management.

Development of a Practical Benchmark for Pig Vocalizations in Noisy Farm Environments

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Acoustic monitoring is a promising, non-invasive approach for assessing welfare and management aspects in livestock systems. Previous studies in pigs have shown that acoustic information can support the detection of welfare-related problems and that vocalizations are linked to the emotional state, discomfort, and stress. Recent approaches use state of the art AI models to extract information on abnormal behavior and critical situations using vocal signals in both unimodal and multimodal settings. However, robust development and deployment in agricultural operations require suitable annotated ground truth datasets, which are currently only available to a limited extent. Pig vocalizations include several call types that differ in structure and context, ranging from low-frequency grunts to high-frequency calls such as squeals and screams. In commercial farms, call assessment is complicated through continuous transitions between call types, overlapping calls, and strong background noise. These factors reduce model performance and complicate the transfer from controlled environments into practice. Although some acoustic datasets are now publicly available and allow training of more advanced AI models, many recordings were collected under controlled conditions and often contain little to no background noise. This limits generalizability and makes it challenging to benchmark models under realistic conditions. Reliable benchmark datasets also depend on an objective sound rating and consistent annotation rules. Single-observer labeling can be subjective, especially when calls overlap or when boundaries between call types are unclear. Therefore, a multi-observer annotation approach can reduce this uncertainty, making inter-observer agreement and intra-observer reliability important indicators of annotation quality and allows the evaluation of systematic difficulties in sound assessment. To address these challenges, the aims of this study are twofold. First, a practical benchmark dataset of pig vocalizations recorded in realistic farm environments is developed, supporting future method development and standardized performance evaluation. Second, an inter-observer analysis is conducted, to improve understanding of call structure and bioacoustic features to identify common obstacles in pig sound assessment. Data were collected on a research farm in Lower Saxony (Germany). Audio and video were recorded in one compartment with six weaning pens and 24 pigs each. For annotation, 30-min sequences from different activity phases were split into 6-s, non-overlapping segments (n=1,500). Five trained observers applied one–zero sampling and assigned presence/absence labels for the classes grunt, squeak, scream, barn noise, machine sound and quiet. Segments were arranged in five overlapping subsets (900 each) so that every segment was rated blindly by three observers. Subsequently, a label was accepted when the observer agreement was ≥2, yielding 1,487 labeled segments (13 unresolved). Inter-observer agreement was evaluated using Fleiss Kappa and was substantial for quiet (0.75), grunt (0.74) and squeak (0.71), and moderate for machine sound (0.62), barn noise (0.55) and the rare scream class (0.58). Mean intra-observer reliability using Cohens kappa ranged from 0.70 (barn noise) to 0.85 (squeak). Overall, this study provides a realistic benchmark dataset recorded in acoustically challenging farm environments, enabling objective evaluation of bioacoustic models and supporting the development of robust on-farm welfare monitoring.

Efficient Bioacoustic Classification of Goat Vocalizations for Edge-Based Precision Livestock Farming

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Advancing on-farm welfare evaluation requires the development and validation of non-invasive, animal-based measures of emotion that can be deployed at scale using precision livestock farming (PLF) systems. While bioacoustics increasingly leverage deep learning on spectrograms or raw waveforms to monitor animal welfare, these models often require significant computational resources and large datasets, making them difficult to implement on resource-constrained edge devices. This study addresses the practical gap in goat-focused welfare monitoring by proposing a lightweight, interpretable alternative that pairs engineered acoustic features with classical machine learning classifiers to reduce compute, memory footprint, and energy consumption without sacrificing actionable performance. This work utilized the publicly available Goat Bleatings dataset from the VOCAPRA project, comprising 4,147 labeled vocalizations sampled at 44.1 kHz across eight distinct husbandry contexts: heat, feed distribution, parturition, injury or death, social isolation, mother-kid reunion, mother-kid separation, and the presence of unknown visitors. A hybrid feature extraction framework was developed to capture spectral, temporal, and bioacoustic descriptors. This included 20 Mel-Frequency Cepstral Coefficients (MFCCs) and their first and second-order derivatives to encode timbral qualities and temporal dynamics. Additionally, the pipeline extracted spectral flux, temporal centroid, spectral contrast, entropy, and ecoacoustic indices such as the Acoustic Complexity Index (ACI) and Bioacoustic Index. Fundamental frequency (F0) statistics were also derived using the pYIN algorithm to provide critical information for distinguishing between high-pitched distress calls and lower-pitched contact vocalizations. An initial screening of 18 algorithms, including tree-based ensembles, linear models, and SVMs, was conducted. The CatBoost Classifier emerged as the top performer with an accuracy of 82.9% and a Cohen's Kappa of 0.793, followed closely by LightGBM and XGBoost. Feature importance analysis revealed a strong preference for MFCC properties, particularly the lower-order coefficients (indices 0–4), which exhibited the highest discriminative variance. While the model faced challenges identifying high-population classes like "heat" and "feed distribution" due to feature similarity, the results demonstrate that this straightforward pipeline achieves an accuracy-to-computational load trade-off that outperforms some heavier convolutional neural network (CNN) implementations for edge deployment. This work helps close the deployment gap between laboratory accuracy and field-ready, low-power monitoring. Future research will focus on a real-time inference pipeline utilizing a sliding window ring buffer and majority voting to reduce temporal "flickering". Furthermore, we propose a multimodal system where the audio engine is gated by visual object tracking, triggering processing only when specific behaviors are visually identified to further optimize edge resources. This work was supported by the "Ayuda a Primeros Proyectos de Investigación" program [grant number PAID-06-25], Vice-rectorate for Research of Universitat Politècnica de València (UPV)

Sound levels during unloading, in lairage and in the stunning area in cattle slaughterhouses

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High sound levels in slaughterhouses can cause stress in animals, impair their willingness to move during unloading or to the stunning area and worsen conditions for rest and recovery in lairage. It is reported that noise levels above 70 decibels (dB) can have negative effects on cow welfare. Others describe thresholds for discomfort in cattle at 90–100 dB. Reducing noise in slaughterhouses is also beneficial for occupational safety and employee health protection, and it is recommended to wear ear protection when exposed to levels above 80 dB. The sound level [dB(A)] was measured using a calibrated sound level meter (Optimus Green CR:171B, Cirrus Research) during unloading, at different locations in lairage and in the stunning area in five medium-sized cattle slaughterhouses in Germany (slaughter rates: 20 to 60 animals per hour). Depending on the size of the slaughterhouse and the number of unloaded lorries/trailers, one to six measurements of up to five minutes each were taken. At one slaughterhouse it was not possible to make measurements during unloading. The measurements were taken during routine slaughterhouse operations, with ongoing slaughtering and unloading of animals, and at the height of or up to one meter above the animals' heads. Sound levels during unloading and in the stunning area were recorded up to a distance of 5 m in front of the ramp or point of stunning. The overall equivalent sound level ranged from 76.9 dB to 81.6 dB during unloading (mean: 79.1), from 74.6 dB to 78.3 dB in lairage (mean: 76.7 dB) and from 77.4 dB to 79.3 dB in the stunning area (mean: 78.6 dB). The maximum peak level was highest during unloading at 123.5 dB, was 118.1 dB in lairage and 116.7 dB in the stunning area. The equivalent sound levels were mostly above 70 dB(A) and in some cases even above 80 dB(A), which can be considered critical for cattle. In addition, it must be taken into account that the commonly used filter 'A' simulates human hearing sensitivity. However, while the frequency of human hearing ranges from 20 Hz to 20 000Hz, cattle can hear frequencies from 23 Hz to 35 000 Hz. Thus, the measurements may underestimate the effect of higher-frequency sounds on the animals. Sounds mainly came from the machines in the slaughter hall, the handling of the animals and equipment by employees, and sometimes from animals' vocalizations. It is recommended to reduce the noise in slaughterhouses in order to improve animal welfare. This could be achieved, for example, with structural modifications such as noise protection between lairage and the unloading/stunning area, use of plastic barriers, gates with rubber stoppers or rubber flooring. In addition, appropriate handling of the animals and barn equipment without yelling and banging is important to reduce noise in slaughterhouses.

Relationship between claw health and increased activity during milking as a supportive trait for lameness detection in dairy cows

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Cows affected by lameness often exhibit increased stepping and weight shifting in order to relieve painful limbs during prolonged standing on hard ground, e.g., at the feeding rack. The objective of this study was therefore to compare the behavior of lame and non-lame cows in milking parlors and to determine whether lameness is associated with increased hind leg movements during milking. Data were collected from 647 cows on 20 Swiss dairy farms. Lameness assessment was conducted during a morning milking as cows exited the milking parlor. Posture and gait were evaluated using a five-point scoring system (1 = normal, 2 = uneven gait, 3 = mild lameness, 4 = moderate lameness, 5 = severe lameness). For statistical analysis, cows with scores of 3 to 5 were classified as "lame," whereas cows with a score of 1 or 2 were classified as "non-lame." Hind leg movements, such as foot lifting, stepping, or kicking, are transmitted as oscillations to the milking cluster and can therefore be recorded using a 3D acceleration sensor. The sensor was attached to the top of the claw of each milking unit. During one evening milking per farm, cluster balancing movements were recorded continuously. Lateral accelerations along the horizontal x-axis were registered as activity when a predefined threshold value was exceeded. This threshold had been determined in a pre-test in which acceleration data from 36 cows (i.e., milkings) were compared with direct behavioral observations of hind leg movements. Individual movements (e.g., foot lifting or kicking) or phases of repetitive movements (e.g., stepping) were counted and expressed as the "number of hind leg activity phases per minute" (HLA) for each cow and milking. However, some milking units exhibited high intrinsic motion, which would have resulted in excessive false-positive detections of HLA. In total, 257 of 647 milkings (39.7%) showed high intrinsic motion and were therefore excluded from the statistical analysis. Linear mixed-effects models were applied with HLA as the outcome variable. Lameness status (two levels: lame, non-lame) and parity (five levels: 1 to 5+) were included as fixed effects. The random effect structure included the individual milking stall nested within farm. Overall, 27% of the examined cows were classified as lame. The statistical model showed a higher frequency of HLA during milking in lame cows than in non-lame cows ($p = 0.026$). HLA also increased with increasing parity ($p = 0.008$). Considerable inter-individual variability was observed, independent of lameness status and parity. This finding may be partly explained by variability between farms, which accounted for 23.9% of the total variance in HLA. In summary, on farm level, lame cows exhibited increased hind leg activity compared with non-lame cows, likely reflecting attempts to relieve painful limbs while standing on hard ground in the milking parlor. Although HLA was measured indirectly via a 3D acceleration sensor attached to the milking cluster and subject to certain technical limitations, automated and continuous recording of HLA during milking shows promise as a tool for early lameness detection. This approach may be particularly valuable for identifying cows with a lameness score of 3, which are often difficult to detect without time-intensive visual assessment.

Image-based classification of horned and polled Caracu cattle using machine learning

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The automated identification of the polled phenotype is particularly relevant for genetic breeding programs, as this trait is of economic interest and may support selection decisions and directed mating strategies. The identification of polled cattle is traditionally performed during calf handling by trained personnel, through visual inspection of horn presence. Polled animals typically present a more rounded head morphology, whereas horned animals tend to show a more angular head shape, which allows visual differentiation between these phenotypes. The objective of this study was to evaluate the feasibility of using image-based machine learning methods to classify horned and polled Caracu cattle based on head morphology, as a non-invasive alternative to conventional identification. A dataset composed of 69 images of Caracu cattle was used, including horned animals (n = 40) and polled animals (n = 29). Images corresponded to a frontal view of the head, focusing on the region above the eyes and between the ears. Image feature extraction was performed using a pre-trained VGG-16 convolutional neural network as an embedding model. The resulting feature vectors were used to train and compare several supervised machine learning classifiers, including k-nearest neighbors, decision tree, support vector machine, Naive Bayes, artificial neural network, and AdaBoost. Model performance was assessed using leave-one-out cross-validation due to the limited sample size. Classification performance was evaluated using accuracy, F1-score, precision, recall, Matthews correlation coefficient, and area under the ROC curve. Among the evaluated models, the artificial neural network achieved the best overall performance (AUC = 0.94; accuracy = 0.86; MCC = 0.70). To further assess model generalization, the best-performing classifier was applied to an independent set of previously unseen images, resulting in 16 correct classifications out of 21 samples. These results indicate that head morphology contains sufficient visual information to allow the classification of horned and polled Caracu cattle using image-based machine learning. However, the use of a larger and more diverse image dataset is necessary in future studies to improve model robustness and generalization performance. This approach shows potential as an automated and non-invasive tool to support animal identification, with possible applications in breeding programs and cattle management systems.

Benchmarking pig detection and tracking under diverse and challenging conditions

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Automated monitoring of individual pig behavior is increasingly important for animal welfare assessment, early disease detection, and understanding social interactions. Video-based systems have great potential to help researchers detect behavioral changes indicating health problems, identify harmful behaviors like tail biting or mounting, and quantify activity patterns over extended periods. However, such systems require reliable automated methods to locate and identify individual pigs across time, and under realistic barn conditions with occlusions, variable lighting, and dust. Despite growing research interest, standardized datasets for comparing different automated pig detection and tracking approaches are scarce, and most existing methods have not been made publicly available. This limits progress toward robust, practical tools that could support behavioral research and on-farm monitoring. To address these limitations, we created two annotated, publicly available datasets: PigDetect (2,931 images from 31 different pen setups) and PigTrack (80 videos with 41 minutes in total from 9 pens), specifically including realistic conditions, such as pigs occluding each other, bad lighting or smudged camera lenses, and rapid movements. We then tested several automated detection and tracking approaches, including both fast methods suitable for real-time monitoring and more accurate but slower methods suitable for detailed behavioral analysis. The key findings were: (1) Including challenging training examples substantially improved detection performance. Specifically, selecting difficult cases for training yielded better results than simply using more randomly selected images. (2) Comparing different detection approaches, we found that state-of-the-art models offer substantial improvements in detection quality over real-time alternatives (85.5% average precision versus 83.6%). (3) For multi-object tracking, we observed that SORT-based methods, which partly rely on hand-crafted association metrics, achieve superior detection performance compared to end-to-end trainable models. However, end-to-end models show better association performance, suggesting they could become strong alternatives in the future. Importantly, methods trained on diverse datasets performed well in completely new barn environments without additional training, demonstrating practical generalizability. When tested on publicly available videos from other research groups, the trained models substantially outperformed more specialized methods, highlighting that carefully curated training data covering diverse conditions may be more valuable than custom-designed algorithms. The detection and tracking methods established here provide foundational tools for behavioral analysis systems. The datasets could be extended with action labels for behavior classification research, with PigTrack particularly suited for this purpose as challenging tracking scenarios include behaviorally relevant interactions like mounting and play. All models, code, and datasets are publicly available, enabling result reproduction, direct application to new data, and farm-specific customization. This supports transparent, cumulative progress in automated livestock monitoring.

Automated Sorting of Finishing Pigs Under Commercial Barn Conditions

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Efficient sorting of finishing pigs is a necessary element in pig production facilities. However, in Canada, sorting is still largely based on manual observation and periodic weighing, which can be labour-intensive, stressful for animals, and difficult to apply frequently in large groups. This study presents the development of a portable, non-invasive AI-based vision system for continuous assessment of finishing pigs under practical barn conditions, with the aim of supporting more precise and timely sorting decisions. The study is being carried out at the swine unit of the Lacombe Research and Development Centre. The imaging platform is positioned at the pen drinking station, where pigs naturally pause in a stable standing posture. An RFID panel reads the ear tags for animal identification and triggers automatic image capture when a pig approaches the waterer. This approach reduces interference from crowding and improves repeatability of image acquisition without altering normal animal behaviour. The sensing system combines two Intel RealSense RGB-D cameras and one FLIR thermal infrared camera connected to an edge computing unit for on-site processing. From these images, phenotypic traits relevant to market readiness are extracted, including body dimensions, body volume, and thermal patterns associated with dorsal fat deposition. Markerless body landmark detection is performed using DeepLabCut, while deep learning models such as EfficientNet and YOLO are being evaluated for live-weight estimation using both publicly available and custom barn datasets. Reference live-weight data are obtained from the barn-installed feed intake and weight recording system. The resulting mean squared error in pose-based landmark estimation ranged from 0.5 to 4.7 cm, while the estimated body weight remained within 5% of the true weight. Preliminary results from the study indicate that continuous phenotyping of finishing pigs in group housing is possible and that by combining estimates of backfat cover, body weight and volume, and feed intake, it is possible to estimate lean meat yield to support carcass-value-based sorting before slaughter.

From counting small ruminants with drones and AI to future applications enabled by offered open data and computer vision models

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"Is my flock complete, or are some animals still out of sight?" This recurring operational question is central to outdoor-based small ruminants farming, where shepherds must frequently verify flock integrity and animal presence, especially at key moments such as gathering, moving animals between paddocks, or bringing them back to shelter. In rangeland systems exposed to predation, this decision is even more critical and can generate substantial stress for shepherds. Conventional approaches, including visual counting and electronic identification, are time-consuming, error-prone, and logistically demanding in real farm conditions, notably because they often rely on handling facilities, and thus rarely implemented. After 2 years of drone testing inside the ICAERUS project, the framework for counting Small Ruminants was described by Lebreton & Helary (2026): focusing on counting animals at key moments and key locations (e.g., along paths, gates, or gathering points) rather than full aerial mapping of entire pastures, in order to reduce technical and regulatory constraints while maximizing counting performance. Our computer vision pipeline relies on 3 core components: (i) small ruminants detection on each video frame, (ii) individual tracking across frames, and (iii) counting through a virtual counting line. We fine-tuned YOLO-based detectors using drone-acquired datasets compiled from open sources and enriched them with additional data to address gaps identified during initial analyses. We targeted data collection in high-density flock flows and for under-represented coat colours (e.g., dark-coloured sheep) that, even at low frequency, occur in many commercial flocks and can degrade detection robustness. For animal tracking and counting on the virtual line, we integrated the algorithm. Detection performance was evaluated on a dedicated dataset (428 images) covering diverse conditions within our defined scope, and assessed the full counting pipeline on three external videos recorded on new farms. The YOLOv8n fine-tuned detection model achieved state-of-the-art performances with 97% precision and 96% recall. End-to-end counting pipeline tests on these new videos further confirmed the capacity to generalize to novel farm conditions, producing counting accuracy considered acceptable by interviewed farmers. This highlights that focusing on key location and key moment improves the overall performance while errors of detection are most likely not located in the virtual counting line and are often not affecting the overall counting performance. Beyond excellent performance, our work highlights the duality of computer vision for livestock farming: high promise, but challenging deployment. Data collection needed for model fine-tuning is slow, annotation is labour-intensive, and real-world variability continues to test model robustness. To help overcome these barriers and accelerate reuse, ICAERUS releases open resources that can support a broader range of applications: more than 4,500 images and 50 videos (with and without bounding-box annotations), representing more than 30,000 small ruminants bounding boxes, along with baseline detection and tracking models. These assets can be reused, fine-tuned, or potentially combined with additional sensors or farm data to build decision-support tools for extensive systems, including animal presence verification, lambing-related alerts, and activity monitoring in outdoor conditions. By making these resources openly available, we aim to lower the entry barrier for researchers and industry developers for robust proof-of-concepts and deployable solutions.

Feasibility of Pulse Oximetry and Infrared Thermometry for Monitoring Vital Signs in Dairy Goats

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Monitoring physiological parameters such as body temperature, heart rate, and respiratory rate is essential for assessing animal health and welfare in dairy production systems. However, conventional measurements, such as rectal thermometry and stethoscope recordings, require animal handling, which may induce stress, compromise animal welfare, and reduce the reliability of physiological data collection. This study evaluated the potential of non-invasive technologies, including an infrared thermometer and a pulse oximeter, to assess vital signs in dairy goats. The experiment was conducted on 56 multiparous Majorera dairy goats in mid-lactation (between the fifth and sixth month of lactation). Measurements were performed in the milking parlour immediately after milking. Infrared temperature was recorded at different anatomical locations and compared with rectal temperature obtained using a digital thermometer. Heart rate and respiratory rate were determined manually using a stethoscope and by visual observation, respectively, and compared with values obtained from a finger pulse oximeter placed on the right teat. The Pearson correlation coefficient was calculated using the CORR procedure of SAS 9.4, and statistical significance was set at $P \leq 0.05$. No correlations were found between rectal temperature and temperatures measured at the horn, mouth, or udder. A positive correlation was observed between anus temperature and rectal temperature ($r = 0.322$; $P = 0.016$). In contrast, heart rate recorded by the pulse oximeter showed a strong positive correlation with manually recorded heart rate ($r = 0.829$; $P < 0.001$). Respiratory rate obtained with the pulse oximeter could not be reliably analysed due to the limited number of measurements displayed by the device. These results indicate that a pulse oximeter may represent a practical non-invasive tool for measuring heart rate in dairy goats under farm conditions. However, additional research is required to identify reliable non-invasive approaches for monitoring body temperature and respiratory rate in this species. Project carried out with a 2025 Leonardo Grant for Scientific Research and Cultural Creation from the BBVA Foundation

Effect of Heat Stress on Heart Rate Variability in Dairy Sheep at Pasture Using a Smartex Textile Sensor

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Climate change represents a major threat to the sustainability of livestock farming, particularly in rural areas where production systems are closely linked to environmental conditions and farm economic viability. Its impact is expected to be especially severe in the Mediterranean area, posing significant challenges to dairy sheep farming systems that are largely pasture-based. Among the main environmental stressors associated with climate change, heat stress is a critical factor affecting grazing ruminants, with potential consequences for autonomic regulation, welfare, and productivity. This study investigated whether heat stress at pasture influences Heart Rate Variability (HRV) in dairy sheep, using the Smartex textile monitoring system. Six Massese dairy sheep were fitted with textile bands and monitored on four summer days (13 May, 31 May, 25 June, and 25 July 2024) for approximately eight hours during the first two days and four hours on the remaining days. During each session, air temperature, humidity, solar radiation, and wind speed were recorded using an on-site weather station, and the Temperature-Humidity Index (THI) was calculated. Flexible regression models accounting for time of day and repeated measures were applied to test the association between Temperature-Humidity Index (THI) and HRV parameters. THI was significantly associated with heart rate ($p = 0.0328$) and RR interval, the interval between successive heartbeats ($p = 0.0384$), showing a non-linear trend. No significant associations were observed for heart rate variability indices reflecting autonomic regulation, including short-term variability and sympathovagal balance. Smoothed effect plots indicated gentle curvature rather than linear responses, suggesting that predicted differences across typical THI ranges provide the most meaningful effect estimates. The findings indicate that Massese dairy sheep exhibit early physiological adjustments to pasture heat stress, reflected by variations in heart rate and RR interval, while maintaining stable autonomic balance. These adaptive responses may represent effective short-term mechanisms that allow animals to preserve homeostasis under moderate thermal load. Further studies on prolonged or extreme heat conditions are warranted to evaluate potential welfare and production impacts, and to assess whether similar patterns are observed in other breeds, which may have different thermotolerance and sheep systems exposed to different environmental and management pressures.

Accelerometer-based behavioural classification in grazing alpacas in the Andes: effects of sensor location and temporal window length

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Behavior monitoring provides indicators of grazing activity, locomotion, and resting behavior associated with pasture use and nutritional status in grazing. Accelerometers combined with machine learning are increasingly used to classify livestock behaviors. However, alpacas exhibit locomotor patterns and grazing behavior that differ from other pseudo-ruminants, limiting the direct application of models developed for other species. This study evaluated tri-axial accelerometers to classify alpaca behaviors in high-altitude Andean production systems. It was hypothesized that accelerometer-derived features enable accurate classification of major behaviors and that sensor placement and temporal window length influence classification performance. Behavioral observations were conducted on six 2-year-old female alpacas grazing in high-altitude Andean production systems in Peru. Eighteen sessions were performed over 3 days in July 2025 (~30 min each) during daytime grazing (10:00, 12:00, and 14:00). Behavior was recorded by continuous focal video sampling, annotated using BORIS® software, and classified into 12 behaviors grouped into four classes: oral activity, locomotion, resting, and mixed activity. Body acceleration was recorded using two tri-axial HOBO® accelerometers on the jaw and forefoot, with signals synchronized to behavioral annotations and resampled to 1 Hz, generating 33,130 labeled observations. Accelerometer data were aggregated using temporal windows of 3, 5, 10, 20, and 30 s. For each window, tri-axial features including mean acceleration, variance, signal magnitude area, energy, overall dynamic body acceleration, vectorial dynamic body acceleration, and mean inclination angles were extracted. Each window was assigned the most frequent behavior within the interval. Classification used Random Forest models (500 trees) trained with features from the jaw sensor, the forefoot sensor, or both sensors combined. Model performance was evaluated using leave-one-animal-out cross-validation with class weights to correct class imbalance. Behavioral observations were unevenly distributed, with oral activity representing most observations, followed by resting, locomotion, and mixed activity. Classification performance increased with window length, with overall accuracy ranging from 0.75 at 3 s to 0.85 at 30 s. Sensor configuration had limited influence: single-sensor models achieved accuracies of 0.75–0.84, while the combined configuration reached 0.85. The best performance was obtained with both sensors and a 30 s window. The Random Forest model achieved a training accuracy of 0.98, while leave-one-animal-out validation yielded 0.85. Tri-axial accelerometers combined with machine learning reliably classify alpaca major behaviors under field conditions, with improved accuracy using both sensors and longer temporal windows. This approach provides a non-invasive tool for monitoring behavior in grazing alpacas in high-altitude Andean production systems. Future research should improve discrimination of individual behaviors and validate the approach across broader animal groups and environmental conditions.

Lambing Detection in East-Friesian Dairy Sheep Using Pen-Level Acoustic Monitoring

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Lambing is a critical phase in sheep farming, associated with high mortality in lambs and ewes. Automatically detecting the onset of parturition may help to improve management of obstetric measures and potentially reduce losses. This study aimed to evaluate whether the onset of lambing in East-Friesian dairy sheep can be predicted using area-specific acoustic activity recorded by permanently installed sensors in lambing pens. Twelve East-Friesian dairy ewes were housed individually in wooden lambing pens (1.3 × 4.0 m) equipped with beamforming microphone arrays (Respeaker, 4mic-array) and video surveillance. Acoustic activity was defined as the frequency of sound events (caused by movement and vocalization) detected within a 45°–90° angular range relative to the sensor plane, with a minimum sound pressure level of −89.5 dBFS. The number of acoustic events varied between pens, but patterns were consistent across them. We found that the acoustic activity increased one hour ante partum and remained higher in the immediate peripartal and postpartal timespan. The data was accumulated hourly, normalized to individual 24-hour baselines, and analyzed using a cumulative sum of differences to the day before. Receiver operating characteristic (ROC) analysis was performed to assess the parameters predictive performance, within 1 hour prior to and in the hour of complete expulsion of the first lamb obtained from the video recordings. Finally, the parameter acoustic activity achieved an area under the ROC-curve of 0.87 (maximum sensitivity: 92%, specificity: 75%) for predicting lambing within one hour before birth. Previous studies on lambing detection using animal-attached sensors achieved good detection rates with reasonable advance warning. Pen-level acoustic monitoring provides a reliable, non-invasive method for lambing detection in sheep, with performance comparable to animal-attached systems. A permanently barn-installed system reduces animal handling, operational costs, and minimizes stress for the animals. On the other hand, the barn-installed approach requires pre-lambing relocation of the ewes to the lambing pens. Future studies should validate the system under commercial conditions across diverse breeds and flock sizes, and assess its impact on perinatal mortality and farm management efficiency. Furthermore, the use of the sensor could be examined in other species and contexts.

Prediction of milk metabolites from mid-infrared spectra and estimation of genetic parameters in dairy goats

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Milk metabolites have been associated with resilience, nutritional stress and energy balance, but their laboratory measurement remains costly, time consuming and difficult to implement in routine. The aim of this study was to predict 13 milk metabolites and one enzyme activity from mid-infrared (MIR) spectra in dairy goats and to estimate genetic parameters for these predicted milk metabolites. Prediction equations were developed for choline, cholesterol, triacylglycerol, glucose, glucose-6-phosphate, galactose, glutamate, malate, free amino groups (NH2), isocitrate, beta-hydroxybutyrate, urea, urate and lactate dehydrogenase activity. The full dataset for prediction included 2,720 milk samples from 160 Alpine goats collected on two geographically distinct experimental farms over three years. To increase milk metabolite variability, goats were subjected to a 2-day nutritional challenge consisting of a straw-only diet during two periods: March in early lactation (mobilization phase) and September in late lactation (repletion phase). The dataset was divided at the animal level into a calibration set (112 goats; 1,820 milk samples) and an independent validation set (48 goats; 900 milk samples). Prediction equations were developed using 212 selected MIR wavenumbers with partial least squares or support vector machine methods. Among the 14 prediction equations, eight showed a coefficient of determination (R^2) greater than 0.64 in validation. Four metabolites exhibited $R^2 > 0.80$: triacylglycerol ($R^2 = 0.81$), cholesterol ($R^2 = 0.84$), isocitrate ($R^2 = 0.85$) and urea ($R^2 = 0.91$). To estimate genetic parameters of predicted milk metabolite concentrations, MIR spectra from routine milk recording collected over seven years on one of the experimental farms were used. The dataset comprised 11,568 MIR records from 880 Alpine goats. Genetic parameters were estimated using a test-day approach with a repeatability animal linear mixed model. The analysis was restricted to the seven metabolites and the lactate dehydrogenase activity because of a validation R^2 greater than 0.64. Heritability ranged from 0.18 (± 0.03) to 0.59 (± 0.04). These heritabilities show the potential of MIR-predicted metabolites for selecting metabolic resilience in commercial farms. The PhD grant of C.B. was funded by Clermont Auvergne Metropole, and the work was funded by the French funding bodies APIS-GENE and CASDAR, under the project names 'Resilait' and 'Casmire', respectively.

A Multi-Stage, Data-Driven Welfare Assessment Framework for Small Ruminants under Intensive Feedlot Conditions in a Hot-Arid Environment

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Animal welfare monitoring across livestock supply chains remains limited in hot-arid production systems, despite its importance for sustainable food production and transparent value chains. The objective of this study was to develop and pilot-test a multi-stage, data-driven welfare assessment framework for lamb production under intensive feedlot conditions and to evaluate the feasibility of translating biological welfare measurements into blockchain-compatible digital records for traceable welfare certification. The framework was applied to 41 Awassi ram lambs (4–6 months of age) raised under an intensive feedlot system with individual pen feeding in the United Arab Emirates. Welfare assessments were conducted across three sequential stages of the production chain: on-farm (feedlot), transport and loading, and immediate pre-slaughter conditions under Halal processing. On-farm welfare was evaluated using standardized animal-based and resource-based indicators structured into four domains: behaviour and mental state, health, nutrition, and environment. Stage-specific indicators were applied during transport and slaughter to evaluate handling, vehicle suitability, and stress-related responses. Welfare scores were generated using a standardized scoring framework and subsequently structured into digitally verifiable data units compatible with blockchain-based traceability systems. On-farm welfare outcomes ranged from moderate to good, with nutrition representing the highest scoring domain, while environmental and behavioural indicators revealed constraints associated with bedding hygiene and restricted movement related to individual housing. Transport and loading conditions achieved a Good welfare classification, characterized by adequate space allowance, appropriate vehicle design, and low incidence of slipping or falling. Immediate pre-slaughter Halal conditions also resulted in Good welfare, with calm handling, minimal vocalisation, and limited behavioural indicators of stress. The study demonstrates that multi-stage welfare assessment can generate structured biological data suitable for digital recording and blockchain-based verification, enabling transparent welfare communication across the supply chain. Such frameworks provide a practical foundation for integrating Precision Livestock Farming data streams, digital traceability, and welfare-based labeling systems in small ruminant production systems operating under hot-arid environmental conditions.

Artificial neural networks outperform partial least squares models for fatty acids estimation using FT-MIR spectrometry on bovine milk: a first step towards transfer learning on small ruminants.

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Fourier transform mid-infrared (FT-MIR) spectrometry in milk can improve production, milk quality and animal welfare among others. Nevertheless, this technology remains underused in dairy ewes and goats, despite it becoming increasingly common in the dairy cattle sector. To reduce the cost of developing equations, transfer learning could be used to adapt FT-MIR models from dairy cattle to dairy sheep and goats. However, this transfer requires artificial neural networks (ANN) but these bovine ANN models do not yet exist. This study aims to develop a multi-layer perceptron ANN for fatty acids to see if we can reach at least the same performance as the ones obtained with commonly built partial least squares (PLS) regressions. The ANN models developed for bovine milk were then directly applied to goat milk to evaluate the potential of transductive learning, the simplest transfer learning method. The database (n=1942) contained 42 individual and groups of fatty acids measured by gas chromatography and their associated standardized bovine milk spectra. A total of 80% was used to train models and 20% used for validation. Validation R^2 values ranged from 0.1223 to 0.9890 for PLS models and from 0.2050 to 0.9886 for ANN models. Compared with PLS models, ANN reduced prediction errors (RMSE) by 0.08 to 47.27% for 39 fatty acids, while an increase in errors ranging from 1.74 to 3.38% was observed for 3 fatty acids. These results highlight an overall improvement in the predictive performance of neural networks compared to PLS models on the validation dataset. After applying the built ANN models on the goat dataset (n=345), only 10 fatty acids have a positive R^2 from 0.1228 to 0.9410, one fatty acid showed an R^2 equal to zero, while the remaining 28 fatty acids have aberrant R^2 values, highlighting the lack of model generalization across species. In conclusion, these results highlight an overall improvement in the predictive performance of ANN compared to PLS models to estimate fatty acids by FT-MIR spectrometry for dairy cattle. Furthermore, transductive transfer learning confirms that models created specifically for dairy cattle cannot be applied as they are to small ruminants and opens the door to testing more sophisticated transfer learning methods.

Early-life milk intake and drinking behaviour as predictors of growth and first-lactation performance in dairy goats

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The pre-weaning period represents a biologically sensitive phase that may influence lifetime productivity in dairy ruminants. While associations between pre-weaning milk intake and later milk yield are well documented in dairy calves, comparable evidence in dairy goats remains limited. This study evaluated the relationships between milk consumption behaviour, early growth and first-lactation milk production using large-scale automatic feeder data. Data were collected from 1,686 Saanen goat kids on a commercial dairy goat farm in the Netherlands equipped with an automated milk feeding system. A total of 361,024 feeder visit records were processed to derive two behavioural indicators during the suckling period: total milk intake and drinking speed. Growth was assessed using Average Daily Gain (ADG) and estimated body weight at 50 days of age (est50). First-lactation milk yield data were available for 1,376 animals. Relationships were analysed using linear regression models ($p < 0.05$). Total milk intake showed a strong positive association with ADG ($\beta = 0.0575$, $p < 0.001$). Drinking speed independently increased ADG ($\beta = 0.0078$, $p < 0.001$), corresponding to approximately 11 g/day higher growth per 1 ml/s increase in speed. Early growth was positively associated with first-lactation milk yield: each additional gram of ADG increased milk yield by 0.0063 kg/day ($p = 0.045$), and each additional kilogram of body weight at 50 days increased milk yield by 0.11 kg/day ($p = 0.030$). This amounts to 40 kg more milk per year, which is around 3.3% and has an impact of €24 per goat per year. Drinking speed showed no direct association with milk yield, indicating that its contribution to adult performance is mediated through growth. No significant relationship was observed between drinking behavior and age at first kidding. Estimated body weight at 50 days emerged as the central biological indicator linking pre-weaning nutrition to adult productivity. Seasonal variation further suggested that environmental conditions and month of birth may modulate early growth potential. In a complementary analysis ($n = 899$), milk replacer formulations differed significantly in ADG. When biological performance was combined with economic evaluation (gain per unit cost), the formulation promoting higher growth also yielded superior economic efficiency, demonstrating that product price alone is not an adequate decision metric. These results demonstrate a clear biological pathway connecting early-life milk intake, juvenile growth and subsequent milk production in dairy goats. Automatic feeding systems provide robust phenotypic indicators for early performance prediction. Future research should determine whether enhanced pre-weaning growth influences lifetime productivity and health outcomes. Additionally, the potential physiological consequences of high drinking speed should be evaluated, particularly whether reduced salivation associated with rapid milk intake may act as a limiting factor for growth performance and overall physiological development. We gratefully acknowledge the support from Urban GmbH and the GIC nucleus farm of Harm and Ankie van der Veen-Meijer for providing the data used in this study.

SNP-guided prediction of feed conversion ratio in Latvian sheep: machine learning models for marker-assisted selection.

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The economic benefit of sheep farmers depends on the quantity and quality of lamb meat produced, which is directly related to the feed conversion ratio. Improving breed selection for feed efficiency would promote the production of high-quality lambs for meat production and breed improvement. Marker-assisted selection (MAS) uses genetic markers associated with specific traits, but these markers must be determined individually for each breed/population. The research seeks to identify molecular markers from the GeneSeek® Genomic Profiler™ Ovine 50K (Lansing, MI, USA) associated with the Feed Conversion Ratio (FCR) in Latvian sheep breeds and to employ these markers in machine-learning-based genomic predictions. DNA genotyping data from 261 controlled fattening lambs across eight prevalent breeds indicate that 135 (51.72%) are of the national breed Latvian Darkhead (LT). The average computed Feed Conversion Ratio (FCR) for the 60-day period (from day 90 to 150) for all lambs was 4.74±0.80 kg, whereas for the LT breed, it was 4.85±0.84 kg. In the Genome-wide association study (GWAS) investigation, one SNP, rs413876950, had a p-value of 3.46 x10-8, alongside 1665 SNPs with $p<0.01$ and 3549 SNPs with $p<0.05$ identified across all breeds. In contrast, the LT breed revealed 478 markers with $p<0.01$ and 1994 markers with $p<0.05$ detected independently. Machine learning methods were employed to integrate lamb birth rates and GWAS significant loci (5215 for all breeds and 2472 for LT) to ascertain the most effective predictors of lamb feed conversion ratio prior to the fattening phase. The resultant MAS prediction would facilitate the identification of prospective breeding candidates before they mature. The results illustrated the predictive powers of machine learning algorithms. The study revealed the potential to integrate machine learning algorithms with genotyping results to enhance breeding efforts in Latvia, particularly for the LT variety, consequently improving breed advancement and competitiveness. Acknowledgments: Funded by UL Faculty of Medicine and Life Sciences internal student grant MG-2026/XXXX, grant No. XXXX and by the Latvian Council of Science, Latvia, projects LZP-2021/1-0489 and LZP-2024/1-0092.

Exploring a data-driven approach to evaluate general resilience in lambs through cumulative feed intake

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Animal resilience is a key concept in livestock production, as more resilient animals are able to better cope with short-term environmental challenges. As resilience is not a directly measured trait, many efforts have been made over the past few years to develop resilience indicators that capture the animals' response to perturbations and may become target traits of selection in breeding programs. In this context, the main objectives of this study were to (1) derive lamb resilience indicators based on variability in cumulative feed intake, (2) evaluate potential differences on resilience phenotypes between sheep breeds and sexes, and (3) investigate the phenotypic correlation between resilience indicators and performance traits in Uruguayan Corriedale, Texel, and Creole sheep. High-frequency individual feed intake data from 439 lambs (176.2 ±47.4 days of age) of three breeds (Corriedale, Creole, and Texel) and offspring of 45 rams (7 Corriedale, 35 Texel, and 3 Creole), collected across seven feeding trials conducted between 2020 and 2024, were used in this study. The animals' average initial body weight (BW) was 37.1 ±9.9 kg for Texel, 30.3 ±7.7 kg for Corriedale, and 22.3 ±2.2 kg for Creole. Feed efficiency trials lasted 53.1 ±6.0 days and animals were conducted at the post-weaning phase. These data were used to estimate target trajectories (in the absence of perturbations) of cumulative feed intake using a smoothing function. Deviations between the observed and predicted cumulative feed intake trajectories were associated with potential perturbations and subsequently used to derive eleven resilience traits. The resilience indicators derived captured different aspects of the perturbation shape, including amplitude, variance of deviation, log variance of deviation, autocorrelation, recovery ratio, recovery time, perturbation time, number of perturbations, average velocity, maximum velocity, and area under the curve. Most of the perturbations occurred at the beginning of the trials (61.9%), which indicates potential challenges involving the adaptation to the facilities. Animals had an average of 1.89 ±0.91 perturbations ranging from one to five across individuals. Differences in estimated marginal means were found for sex in the Texel breed, where males were generally more resilient. Additionally, differences were found between the three breeds inside the male group, where Creole male lambs exhibited improved resilience as compared to Texel and Corriedale. Unfavorable phenotypic correlations were observed between some resilience indicators and production traits such as average daily gain and rib eye area. A favorable correlation was observed between resilience and fat thickness which suggests that fat deposition plays an important role in adaptation and resilience. No unfavorable association was found for residual feed intake, suggesting that, at a phenotypic level, an increase in feed efficiency does not affect animals' resilience. Results indicate that this data-driven approach is a useful tool to evaluate animals' trajectory and responses to unknown environmental challenges. The phenotypic correlations highlight an anticipated pattern of potential trade-offs between resilience and production that warrants further investigation based on genetic analyses and larger datasets. More balanced breeding strategies will likely be needed when selecting for more productive animals.

Combining multi-sensor data and machine learning for automated behaviour classification and disturbance detection in sheep

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Sheep production systems are frequently considered low-input and robust, particularly under extensive pasture conditions. However, intensive grazing dynamics, seasonal fleece growth, and the high incidence of lameness, injuries, and predation events require continuous oversight to safeguard welfare and productivity. In self-managed pasture systems, animals may not be visually inspected on a daily basis, increasing the probability that health disorders, mortality, or abrupt behavioural disturbances remain undetected. Early identification of such events is critical for welfare assurance, economic stability, and adaptive predator–livestock management in regions with increasing large carnivore presence. The aim of this study was to integrate multi-sensor technology and algorithm-based behavioural inference in group-housed, pastured sheep. As a result, the high-quality sensor data yielded a comprehensive characterization of small ruminant behaviour. Particular emphasis was placed on detecting rapid, synchronized movement bursts, spatial dispersion events, and orientation shifts indicative of external disturbances such as predator encounters or acute stress responses. Therefore, individual behaviours as well as herd dynamics were considered. Data were collected by equipping eight sheep with VERTEX Plus collars (Vectronic Aerospace GmbH), a multi-sensor system recording tri-axial acceleration, GPS position, magnetometer-based orientation, and proximity to conspecifics at high resolution. Simultaneous direct behavioural observations provided ground truth for supervised model development. Based on these synchronized datasets, a random forest classifier was trained to distinguish core behavioural states (rumination, grazing, resting, locomotion, vigilance, and social interaction) and to identify sudden deviations from established activity patterns. Initial model validation indicates clear separability of primary activity states and promising potential for identifying abnormal movement clusters. Individual deviations may indicate reduced welfare, whereas herd-level deviations may reflect external disturbances. Further work will establish automated scoring coupled with real-time alert functions. The envisioned system architecture aims to notify shepherds of welfare-relevant anomalies such as injury or predator threat and enable rapid on-site intervention. In addition, this approach supports evidence-based livestock-wildlife-coexistence strategies by objectively documenting disturbance events. Ongoing work focuses on evaluation across alternative sensor configurations and parameter optimization throughout the pipeline of data analysis. Practical implementation of the system must further address requirements for data transmission, and scalability across diverse pasture systems. The project is supported by funds of the Federal Ministry of Agriculture, Food and Regional Identity (BMLEH) based on a decision of the Parliament of the Federal Republic of Germany via the Federal Office for Agriculture and Food (BLE) under the innovation support programme.

Assessment of Optimum Drying-Off model on Milk Yield in Assaf Ewes

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Drying-off in the dairy sheep industry is an important management practice with impact on productivity and farm profitability. Proper timing of drying-off allows the mammary gland to recover before the next lactation and can influence subsequent milk yield. Therefore, optimizing the drying-off process may improve production performance while reducing potential economic losses. Additionally, this process influences the health and welfare status of animals, which has become a key aspect in modern production systems. The aim of this study was to explore, using regression analysis, the effect of drying-off timing on subsequent total milk yield in Assaf dairy sheep and, consequently, to determine the optimal drying-off timing as a recommended management practice. To achieve this, a mixed model was applied to account for both fixed and random effects in the analysis. The analysis was based on 152,609 milking records from 52,263 ewes belonging to 36 Assaf dairy farms in Castilla y León, Spain, collected between 2001 and 2023. Records were collected by the Spanish Assaf Sheep Breeders' Association (ASSAFE) as part of routine milk recording activities. After filtering out outliers, 149,003 milking records from 52,615 ewes remained for the analysis. Multiple nonlinear mixed models were fitted to explain the total milk yield after drying-off (TMY2). Variables considered in the analysis were: drying-off days before parturition (DRY), TMY1 (total milk yield prior to TMY2), lambing season (LS), drying-off season (DS), and number of lambings (NL2 = 1 vs. ≥2 parturitions), included as independent variables; and farm (FA), ewe nested within farm (FA:EWE), and lambing year (LY), included as random effects. TMY1 was standardized using the scale function (TMY1z) and subsequently categorized into two levels: high and low total milk yield (TMYLH). The DRY variable was modeled using nonlinear functions, including natural splines and orthogonal polynomials implemented in the splines and stats R packages. Multiple non-linear mixed models were fitted using the lme4 package in the R environment. An ANOVA test was performed to compare models and assess statistical differences, while model fit was evaluated using the Akaike Information Criterion. The analyses indicated that the best-fitting model included the effects:TMY2 = ns(DRY, df = 9) × DS + TMY1z + LS + DS + NL2 + FA + EWE(FA) + LY + Error. This model suggests that the interaction between DRY and DS improves the accuracy of predicting the most suitable drying-off timing, indicating that management decisions regarding drying-off should not rely solely on the number of days but also on the season in which drying-off occurs. Thus, the optimal drying-off point differs across seasons, ranging from slightly more than 50 days for ewes dried in winter and spring to approximately 200 days when drying occurs in summer. These results should be interpreted in the context of the seasonality of sheep reproduction, and more specifically within Assaf production systems in Spain. Overall, the results of this analysis show that non-linear mixed models can be an effective tool for determining optimal management practices for drying-off in dairy sheep, thereby maximizing the total milk yield of subsequent lactations using routinely collected production data, as illustrated in this case with Assaf flocks in Spain.

Mineral requirements of Tenebrio molitor larvae: magnesium, potassium and phosphorus needs

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Minerals represent a minor proportion of livestock diets; however, they are indispensable for optimal livestock health and performance. Deficiencies in mineral intake may result in impaired growth and reproductive issues, consequently, the mineral requirements of livestock are well documented. In contemporary legal contexts, insects are categorized as farmed animals. However, their rearing practices typically involve the utilization of substrates designed for other species, such as chicken feed, or circular substrates composed of by-products that are not balanced in their nutritional composition. There is limited knowledge about the nutritional requirements of insects, and improvements can be made to their substrates to maximize their performance. It has been demonstrated that, when sufficient protein is provided, the amino acid concentration of the substrates had a limited influence on the growth performance of mealworms (Tenebrio molitor) (MW), but their mineral requirements have not yet been investigated. Therefore, the present trial aimed to analyze the mineral needs of MW. The focus was on the requirements of magnesium (Mg), potassium (K) and phosphorus (P), based on the mineral content of wheat bran, the gold standard for rearing MW. Semi-artificial diets based on at least 25% wheat bran supplemented with sucrose, casein and inorganic minerals were tested. The diets were isoenergetic and isoproteinous. Each mineral was administered in four different doses per experiment (from 25% to 100% of the mineral content of wheat bran) and the other minerals were given in inorganic form at a concentration of 100%. The growth performance of the MW larvae was measured using two key metrics: weight gain and survival. The collected data were then analyzed in SPSS using a univariate ANOVA for final total weight and survivability, and a linear mixed effects model for individual larval weight measured over time. The groups demonstrated a comparable survivability rate (>90%), with the exception of the Mg 50% and Mg 75% groups, which exhibited a numerically lower survivability rate of 89.25%. Considering the average larval weight, differences have been observed between the different mineral groups. Specifically, the K 25% group demonstrated a lower mean individual weight in comparison with the Mg 50%, Mg 75%, P 50%, P 75% and P 100% groups. K 75% larvae showed a lower individual weight in comparison with Mg 75%. The differences observed were partially confirmed in the final total larval weight. Indeed, the K 25% group showed a significantly lower value in comparison with most of the other groups. These results indicate that several minerals are supplied in excess to MW using traditional substrates, and that a quantity higher than 25% of the K present in wheat bran seems to be crucial for MW growth. However, further studies are needed to understand the observed growth differences and to design a substrate that completely fulfils the nutritional requirements of MW.

Effects of structural fibre ingredients on black soldier fly (Hermetia illucens L.) larval growth at industrial and laboratory scales

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Optimising structural fibre components for Black Soldier Fly larval (BSFL) diets is essential for scaling insect-based bioconversion systems. This study evaluated the impact of three fibre-rich materials, reed-ditch grass mix, nature straw, and sawdust (control) on BSFL growth and composition. The reed–ditch-grass mix and natural straw were composted with ammonia-washer fluid on a small scale, while sawdust was used untreated; all materials were incorporated at approximately 20% of the total substrate mass. Trials were conducted under industrial crate conditions using 55,000 four-day-old larvae per replicate (n = 6) over a six-day rearing period. The larval growth performance and biochemical profile were analysed. A complementary laboratory-scale experiment (200 larvae per replicate; n = 5) was performed to confirm substrate-specific performance trends and assess additional fibre variants composted at a larger, industrial scale. Although the diets differed only slightly in biochemical composition, their structural properties had a clear impact on larval performance outcomes at the large-scale. Larvae reared on the reed-ditch grass mix and on the sawdust control achieved the highest total larval biomass (both 4.33 kg), while nature straw yielded the lowest biomass (3.45 kg). Individual larval weights showed the same pattern, with reed-ditch grass producing the heaviest larvae (89.7 mg), followed by nature straw (84.0 mg) and the control (75.2 mg). The survival rate ranged from 75% (nature straw) to 100% (control). Residual substrate weight also varied, with nature straw leaving the highest residue. The larval biochemical composition remained relatively consistent across treatments. Crude protein content (N×4.76) ranged from 8.7% in larvae reared on the reed-ditch grass mix to 9.1% on nature straw, while fat content varied from 1.88% (reed–ditch grass mix) to 2.10% (control diet). In the small-scale experiment, the measured parameters, including larval final weight, survival, and frass output, confirmed the substrate-dependent growth trends observed at the large scale. The survival rate was above 95% for all diets. The highest harvested larval biomass and individual larval weight were for larvae reared on reed-ditch grass mix (22.1 g and 111.8 mg, respectively) and reed alone (21.9 g and 109.8 mg, respectively). Together, the two experiments demonstrate that fibrous substrates can support robust BSFL growth, but their effectiveness varies with fibre type and composition. Reed-based diets showed the most consistent performance across scales, highlighting their strong potential for industrial application.

Protein-to-soluble carbohydrate ratio in the diet for Tenebrio molitor imagoes modifies their reproduction, egg viability and young mealworm survival

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Mealworms (Tenebrio molitor larvae) are one of the most relevant species for feeding birds, multiple captive reptiles, amphibians and even mammals due to their high nutritional value for insectivores. To optimise industrial mealworm production, it is essential to maximise egg production and viability, as well as further mealworms survival. However, information on the nutrition of imagoes to optimise egg production is limited. The aim of this study was to evaluate the effect of different protein-to-soluble carbohydrate (P:SC) ratios on the development of T. molitor imagoes, their egg-laying productivity and further survival of the young mealworms. To this end, four experimental diets with the following P:SC ratios were designed: 1:1.7, 1:1, 1.7:1 and 1.7:1F (the latter of which contained 2% fat-F), changing the proportion of wheat bran, soyabean meal and soyabean oil, but maintaining the carrot content. A total of 20 60×40 cm trays (5 per diet) with 350 g of imagoes housed at 27ºC and 70% relative humidity were used for 24 days. The feed intake, weight and mortality of the imagoes during the reproductive period was monitored two times per week, as well as the oviposition and hatching rates of the eggs and the survival rate of the young mealworms during the first month of life. Results indicate high-protein diets (1.7:1 and 1.7:1F) reduced the dry feed intake of imagoes, but they significantly increased the intake of wet feed (carrot) and of the total diet. This is probably due to the greater water requirement needed to process excess nitrogen and form protein retention. Although no significant differences were observed in average daily weight gain, imagoes fed a high-protein, high-fat diet (1.7:1F) reached a higher final weight, suggesting greater nutrient accumulation. Furthermore, these higher-protein diets increased egg weight linearly (by +9.5% from a P:SC ratio of 1:1.7 to 1.7; $P<0.001$), although decreased daily egg production (by -10% from 1:1.7 to 1.7:1 P:SC ratio; $P<0.001$). This trend was partially reversed with the inclusion of fat. The age of imagoes also affected egg production and size. With increasing imago's weight, egg weight also increased by +0.014 mg per day ($P<0.001$), but daily egg production decreases by -0.52 eggs per female per day ($P<0.001$). There were no significant differences in the hatching rate of the eggs depending on the diet provided to the imagoes (on average 84%). With regard to the mortality of young mealworms during the first month of life, mealworms from imagoes fed the 1:1 diet showed greater mortality than those fed the other diets (on average +3.11 percentage points; $P<0.05$). These results suggest that, depending on the production objective, the optimal protein-to-soluble carbohydrate ratio could differ. Richer protein diets promote heavier eggs, while diets with a higher energy content increase the number of eggs obtained. In addition, the diet supplied to the imagoes affects the survival of the mealworms in the early stages of their life.

The effect of tomato leaf waste handling on the growth performance, nutritional composition, and glycoalkaloids content in the desert locust Schistocerca gregaria

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Abstract^Tomato leaves are an abundant agricultural waste stream, but their utilization as feed is limited due to their high fiber content and the presence of toxic glycoalkaloids. However, using tomato leaves as feed for desert locusts can be a feasible approach to produce insect protein for food and feed applications. In this study, desert locusts were reared on freshly harvested leaves, leaves stored for varying durations, and on leaf cuttings kept in water to maintain freshness. Feeding on leaf cuttings kept in water reduced α-tomatine and dehydrotomatine contents in the locusts, despite the high alkaloid content in the leaves. The chemical composition of the produced locusts varied across treatments, with the leaf cutting method resulting in higher accumulation of Cu, Zn and Pb compared to feeding with fresh leaves. The crude protein content of the locusts remained stable across treatments (59.37–60.51%), so was their fatty acid composition. These findings highlight the impact of leaf storage conditions on the production performance of desert locusts, which is crucial for developing an efficient and low-labour entomoponic cultivation system.

From olive waste to insect biomass: evaluating increasing levels of olive pomace in black soldier fly production

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Several studies on black soldier fly (Hermetia illucens; BSF) have demonstrated the suitability of agro-industrial residues as substrates for larval development. However, many studies evaluated heterogeneous substrates, making it difficult to isolate the specific effect of individual by-products on larval performance. Experimental approaches using increasing inclusion levels of a single by-product within a constant basal diet allow a more precise evaluation of ingredient suitability. Additionally, most trials are conducted at laboratory scale using relatively small quantities of substrate, whereas rearing scales may influence larval density and substrate conversion efficiency. The objective of the present study was to evaluate the effect of increasing inclusion levels of olive pomace on larval survival and substrate conversion performance of BSF under pilot rearing conditions. Six experimental diets were formulated with increasing olive pomace (0, 10, 15, 20, 25 and 30%; named D0, D10, D15, D20, D25, D30, respectively), while maintaining similar composition for the remaining ingredients (brewers spent grain and yeast, eggs, whey, dry and liquid distillery stillage, rice husk, water, breading and vegetable waste). Each treatment was replicated four times (10kg of substrate/replicate, 14,000 6 days old larvae [average weight: 8.7 mg ± 0.169]/replicate). Larvae were reared at 28°C and 60% RH. The end of the trial was set when 3 replicates/treatment reached at least 5% of prepupae. After that, the larval biomass and residual substrate weight were recorded and the following indicators were calculated: reduction rate (RR), waste reduction index (WRI) and bioconversion efficiency corrected for residue (BER). Larval average weight and survival rate (SR) were also estimated. Data were analyzed using One-Way ANOVA (SPSS, v28; $P<0.05$). The larvae of D0 reached the highest final average weight ($P<0.001$), followed by D10 and D15 that were statistically equal ($P>0.05$). No differences were observed among D15, D20 and D25 ($P>0.05$), and the last two groups were also equal to D30 ($P>0.05$). Olive pomace inclusion also affected larval survival, with the D20, D15 and D10 treatments showing higher SR than D30 and D25 ($P<0.001$), while D0 was intermediate and comparable only to D15 ($P>0.05$). The highest RR was observed in D30 ($P<0.001$) and the lowest in D10 ($P<0.001$), which was statistically equal to D0 ($P>0.05$). The best WRI was recorded in D0 and D10 ($P<0.001$). In contrast, D25 and D30 showed the poorest values ($P<0.001$), while D15 and D20 showed intermediate values ($P<0.001$). This outcome may be referred to the development time that was 8 days for D0 and D10, 10 days for D15, 11 days for D20, 15 days for D25 and 16 days for D30. The D0 and D10 displayed the highest BER, while D25 and D30 showed the lowest values ($P<0.001$). In conclusion, moderate olive pomace inclusion levels (up to 15%) provided the most balanced performance in terms of larval survival, growth and bio-conversion performance. Although higher inclusion levels improved waste reduction, they negatively affected larval viability, maybe due to the phenolic compounds present in olive pomace. The research is part of the OLIWA project, founded by the PRIMA program supported by the European Union's Horizon 2020 Research and Innovation program under Grant Agreement N2432

Integrating Insect Production Into Greenhouse Systems: Using Tomato Leaf Biomass as Insect Feed

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The Closed Urban Modular Energy- and Resource-Efficient Agricultural System (CUBES), built at the Humboldt University of Berlin (Germany), integrates insect production into a circular production model alongside fish and plant cultivation, mirroring natural ecosystems. The presented work focuses on establishing nutrient synergies between plant (greenhouse) production and insect production systems mainly by exploring the potential use of tomato leaves as feed for insect rearing. Experiments were done using different insect species: the black soldier fly (Hermetia illucens), yellow mealworms (Tenebrio molitor), desert locusts (Schistocerca gregaria), and the tobacco hornworm (Manduca sexta). Tomato leaves commonly grown in greenhouses are not suitable for black soldier fly or mealworm rearing, with negative effects even with partial usage. However, these effects are variety-dependent and differ based on the toxic glycoalkaloid content of the tomato leaves. Herbivorous insects such as the desert locust and the tobacco hornworm can consume and grow efficiently on these leaves but toxic glycoalkaloids can be present in their biomass. The content of toxic plant glycoalkaloids can be reduced with proper downstream processing. A comparison between the production of desert locusts and the tobacco hornworm using tomato leaves as feed will be presented, in addition to challenges in their rearing.

Turning Waste into Value: Effects of olive fragments on Black Soldier Fly Larvae Performance

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Olive oil production generates significant amounts of agricultural by-products, particularly in Mediterranean regions. Among these residues, olive fragments, consisting mainly of leaves and small branches, remain largely underutilized despite their potential nutritional value. Black soldier fly larvae (BSFL) are known to efficiently convert organic residues into valuable biomass. Thus, the aim of the present study was to evaluate the effects of different inclusion levels of olive fragments in BSFL diets on their growth and overall performance. Diets were formulated to fall within the reported protein (16–20%) and lipid (2–6%) requirements of the larvae , with the exception of the diet containing the highest level of olive fragment inclusion, in which the protein content was slightly lower. More specifically, the control diet consisted of wheat bran, whey, and brewer's spent grain. Based on this formulation, three additional diets were prepared by incorporating olive fragments at different inclusion levels: 5%, 12%, and 25%. Larval weight and survival were recorded every three days, and the experiment was terminated when 10% of the larvae had reached the prepupal stage. At the end of the experimental period, Feed Conversion Ratio (FCR) and Bioconversion Ratio (BCR) were calculated, while the nutritional composition of both larvae and diets was determined through chemical analyses. Statistical analysis revealed no significant differences in survival rates, which remained higher than 90% across all dietary treatments. In terms of larval performance, the control diet and the 5% inclusion diet yielded the most favorable results, whereas the 25% inclusion diet resulted in the poorest performance. The diet containing 12% olive fragments exhibited intermediate performance. Furthermore, nutritional analyses indicated high protein levels, ranging from 50% to 56%, as well as satisfactory lipid content (2–9%) for larvae reared on the control and 5% inclusion diets, respectively. Overall, the results suggest that BSFL can tolerate the inclusion of olive fragments in their diets at low to moderate levels without negatively affecting feed efficiency or larval performance. Acknowledgement: This research is supported by the EU-PRIMA program – project OLIWA-Repurposing OLIve WAste in circular economy solutions for feeds, additives, packaging, and biogas – GA 2432, Call 2024 Section 1 Food Value Chain in Nexus.

Valorizing Apple and Olive Pomaces: How Density and Substrate Shape Hermetia illucens Larval Performance

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Vast quantities of agro-industrial wastes are generated every year and, in many cases, proper management is limited or entirely absent. Apple and olive pomaces are seasonal by-products produced during apple processing and olive-oil extraction, respectively. Although black soldier fly (BSF) larvae have been widely studied, the combined effects of larval density and diet on their performance remain unclear. This study examined how different larval densities and substrates influence BSF growth and development. Four iso-nitrogenous and iso-energetic diets were prepared using brewer's spent grains, wheat bran, apple pomace, and olive pomace. The first diet contained 30% apple pomace (30AP) with brewer's spent grains and wheat bran. The second contained 30% olive pomace (30OP) with the same base ingredients, while the remaining two diets included both apple and olive pomace along with wheat bran and brewer's spent grains. Three larval densities were tested (1, 2.5, and 5 larvae/cm²). Larval weight and survival were monitored every three days, and the trial ended once 10% of larvae reached the prepupal stage. Chemical analyses were conducted to determine crude protein, crude fat, and ash, and feed performance indicators such as feed conversion ratio (FCR), waste reduction rate (WRR), and specific growth rate (SGR) were calculated. The results showed clear differences among treatments across most parameters, except for survival that remained above 90% for all treatments. The highest density (5 larvae/cm²) showed the fastest development on the 30AP diet, whereas the 30OP diet showed the longest development time for all densities. Individual larval weight also varied significantly across diets and densities, with 30OP having the lowest weight across all densities. Protein content exceeded 45% in all cases, with the highest value being recorded for the 30AP diet. Overall, the density of 5 larvae/cm² for all diets appeared to be the most favorable under these conditions, suggesting that larval density and diet strongly influence outcomes. Acknowledgments: This research is supported by the EU-PRIMA program – project OLIWA- Repurposing OLIve WAste in circular economy solutions for feeds, additives, packaging, and biogas – GA 2432, Call 2024 Section 1 Food Value Chain in Nexus.

The effect of the growth substrate on the content of essential amino acids in Hermetia illucens larvae

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The black soldier fly (Hermetia illucens) is a widely distributed insect that would be considered a novel food for human consumption. The species is used for food waste disposal, contributing to greenhouse gas reduction, and is considered an alternative nutrient source for farm animals. The chemical composition of the substrate on which Hermetia illucens larvae are grown determines their chemical composition and biological value. Therefore, this study aimed to investigate how different substrates affect the essential amino acid content of the larvae. Five groups of at least 1,000 larvae were reared in separate 90-litre containers containing different food industry waste substrates with no commercial value. Different substrates have been used: 1 – vegetable and fruit waste (potatoes and apples); 2 – grain (wheat and corn); 3 – raw chicken processing waste (intestines, skin and internal organs); 4 – mixture of raw pork and beef by-products (intestines, skin and internal organs); 5 waste river fish. The substrates differed in chemical composition. Vegetable and fruit substrate had high moisture (82%) and low protein (1.2%), while grain substrate had low moisture (9.9%) and high carbohydrates (72.8%). Animal by-product substrates (groups 3–5) contained higher protein (19–22.4%) and fat (11.1–20%) levels. Larvae samples were analysed for amino acid composition using capillary electrophoresis. Statistical analysis was performed using ANOVA with Duncan's multiple range test at $p \leq 0.05$. Results showed that larvae grown on chicken by-products (Group 3) had the highest total essential amino acid content (23.15 mg/100 g). Their total content significantly exceeded that of larvae from Groups 5, 4, 2 and 1 by 0.37, 0.57, 0.63 and 3.83 mg/100 g, respectively. Group 3 larvae also had higher levels of isoleucine, leucine, tryptophan and arginine compared to other groups. Larvae grown on grain substrate (Group 2) had the highest phenylalanine, methionine and histidine content. Vegetable and fruit substrate (Group 1) resulted in the highest threonine content. Pork and beef by-products (Group 4) produced larvae with the highest valine content. Larvae grown on waste river fish (Group 5) did not show the highest value for any amino acid, but their total essential amino acid content was second only to Group 3. The study demonstrates that substrate composition significantly influences the essential amino acid profile of Hermetia illucens larvae. To obtain larvae with the highest overall essential amino acid content, poultry slaughterhouse waste is recommended as a substrate. Moreover, substrate composition can be modified to produce larvae with a targeted amino acid profile.

Dietary fat sources modulate growth performance and lipid composition of black soldier fly (Hermetia illucens) larvae

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The growing demand for sustainable fat sources in animal nutrition has increased the need for alternatives to conventional vegetable oils such as palm oil, whose production is associated with substantial environmental impacts. Insect-derived lipids from black soldier fly larvae (BSFL, Hermetia illucens) represent a promising option because larvae efficiently convert low-value organic side streams into protein- and fat-rich biomass. The fatty acid composition of BSFL fat influences energy supply and exerts functional effects, such as antimicrobial activity, making it a strategic component for animal feed formulations. Yet, the mechanisms by which dietary fatty acids modulate lipid deposition and fatty acid composition in BSFL remains poorly understood. A total of 420,000 six-day-old larvae were reared under controlled conditions (28 °C; 55 % rH) for 8 days and assigned to five isonitrogenous diets containing 6 % fat (DM basis) from beef tallow (BT), olive oil (OO), sunflower oil (SO), or linseed oil (LO), or a Gainesville diet (GD; 1.8 % fat, DM basis) (n = 6). Feed and larvae were analyzed for gross energy, fat, nitrogen, ash, and fatty acid composition. Diet significantly affected BSFL performance. BSFL fed the GD diet showed the lowest average daily weight gain (201.2±6.4 g/d/box; P=0.024) and survival rate (87.2±1.6 %; P<0.001), while BSFL fed BT had the highest values (245.7±4.7g/d/box; 96.3±2.2 %). Regardless of diet, the fat composition of the BSFL was characterized mainly by lauric acid (LA) and other saturated fatty acids (SFA), which were synthesized de novo, as LA was not present in any of the five feed substrates. BSFL reared on SO and LO substrates showed the lowest level of SFA (SO:46.4±3.1 and LO:48.1 ±3.7 g/100 g of total lipids, respectively, P<0.001), while the inclusion of animal-based fat in the diet (BT) increased the SFA level (62.3±2.5 g/100 g of total lipids). Larvae fed SO and LO had the highest (SO:23.0±0.2 and LO:24.2±0.7 g/100 g of total lipids; P=0.004) levels of polyunsaturated fatty acids (PUFA), whereas larvae fed BT and OO had the lowest (BT:11.2±1.4 and OO:15.3±0.9 g/100 g of total lipids). However, there were no significant differences in GE, fat or nitrogen concentrations among larvae fed diets with added fat. Ash content was highest in BSFL fed GD and lowest in BSFL fed BT (9.1±0.3 and 6.2±0.5 g/kg DM; P<0.001). These results demonstrate substantial metabolic plasticity in BSFL lipid metabolism. Dietary fatty acids can therefore be used to strategically modulate insect lipid composition, enabling the targeted production of insect-derived fats for sustainable feed formulations.

Trentinsect: insects and sustainability in feed production

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One of the future challenges for the agri-food sector will be to reduce the waste it generates. The combination of reutilisation of unutilised by-products and waste streams with the creation of more sustainable agro-industrial systems allows to reduce the environmental footprint of the food and feed production. Insect bioconversion is a perfect candidate for circular economy systems, by absorbing organic waste and byproducts and converting them into animal protein. The Trentinsect project is the result of a collaboration between the industrial and academic sector: the University of Trento (Italy), and the company Kinsect. The project aims to develop and optimise, within a circular economy framework, the mass rearing of the black soldier fly (Hermetia illucens) larvae using locally available plant-based residues and agro-industrial byproducts from the Trentino-Alto Adige region, in the north of Italy. The objective is to identify and characterise agro-industrial byproducts and plant-derived waste streams into optimised multi-ingredient diets that meet larval nutritional requirements with the ultimate goal of producing sustainable protein ingredients for animal feed. Locally sourced substrates will undergo comprehensive physicochemical characterisation, including nutrient profiling, assessment of dry matter, granulometry, viscosity, water retention capacity, and contaminant screening. These data will support the formulation of scalable diets, which will be validated through experimental trials evaluating larval growth kinetics, substrate reduction efficiency, bioconversion rates, and biomass nutritional quality. To ensure year-round operational feasibility, innovative storage and stabilisation strategies for seasonal byproducts will be developed. The project will compile a structured database of substrate characteristics and establish a predictive substrate performance impact model linking feed formulation, larval productivity, economic viability, and environmental footprint indicators, thereby enabling to estimate technical and economic outcomes based on locally accessible resources. By improving the management of organic residues, reducing waste disposal burdens, and enhancing resource efficiency, the project aims to strengthen the sustainability of the local agri-food sector.

Use of ergot (Claviceps purpurea) - contaminated rye in the rearing of black soldier fly (Hermetia illucens) larvae

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Sustainable protein production depends on the valorization of biomass streams that are excluded from conventional food and feed chains. Ergot-contaminated grain represents one such underutilized resource, caused by the fungal pathogen Claviceps purpurea, which proliferates under humid conditions during flowering and harvest, as observed in Germany in 2024. Contaminated rye cannot be processed for flour or animal feed due to toxic ergot alkaloids (EA), even at low concentrations. Black soldier fly larvae (BSFL, Hermetia illucens) exhibit broad substrate plasticity and high bioconversion efficiency, transforming heterogeneous organic residues, including manure and agro-industrial by-products, into protein- and lipid-rich biomass. Yet their ability to tolerate or accumulate EA remains largely unknown. This study investigates whether dietary inclusion of ergot-contaminated rye affects larval growth, biomass yield, and EA accumulation in BSFL. A total of 336,000 7-day-old larvae (DOL) with an initial larvae weight of 6 mg/larvae, were allocated equally to 4 treatments with six replicated each (14,000 larvae per box (40x60cm)). Larvae were reared for 8 days in a climate chamber under controlled conditions (28-30 °C, 55-65% rH). The basal diet consisted of 50% rye, 40% wheat bran, 7% concentrates, and 3% apple pomace. Treatments differed in ergot contamination level in the rye: T1 (CON) contained uncontaminated rye and formed the control group. T2 (LOW) contained contaminated rye with ergot bodies removed by sieving, T3 (MID) contained low contamination (0.67 g ergot/kg), and T4 (HIGH) contained high contamination (2.34 g ergot/kg). Larval growth was monitored every two days by weighing subsamples of 50 larvae per replicate. Substrate pH and temperature were recorded at the same intervals. Composite samples of rye, substrate, frass, and larvae (DOL 15) were analyzed for EA content. Data were analyzed using one-way ANOVA ($p<0.05$) followed by post-hoc-Tukey HSD. After 8 days of trial, no significant differences in larval growth performance were observed among the treatments. MID-fed larvae showed the highest weight (155 mg/larvae), while HIGH-fed larvae the lowest (144 mg/larvae, $p>0.05$). A similar trend was observed for biomass yield. The dietary treatment had no effect on biomass yield; however, LOW was numerically higher (2.18 kg larvae FM/box), while HIGH was the lowest (1.96 kg/box; $p>0.05$). Dietary ergot inclusion had no effect ($p>0.05$) on larval survival rate. EA were detected in rye, substrate, and frass but were not detected in larval biomass. In conclusion, the inclusion of ergot-contaminated rye (up to 50% of the diet) appears to be a suitable feed for BSFL without compromising performance or resulting in detectable transfer of EA into larvae. Further research is required to confirm these findings and assess long-term effects. Acknowledgement: The study was conducted as part of Project BSFL meets MycoGrain and financed by the Ministry of Economics, Transport, Agriculture, and Environment of Rhineland-Palatinate, Germany. We would also like to thank DSM Nutritional Products GmbH (Dr. Wolfgang Markert) for supporting the mycotoxin analyses.

Methodological approach for multi-trait information pooling into single-trait using deregression: a study case on boar taint

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The current study proposes a pre-treatment designed to condense information from a multi-trait (MT) model into a single reference pseudo-phenotype that can be used in a single-trait (ST) model for routine evaluation. This need arises from the current context of selection, which is constantly evolving by incorporating an increasing number of new traits (i.e., emergent traits) to characterise complex phenotypes that can be costly to measure, heterogeneous, poorly heritable, or limited in availability, complicating MT analyses. For example, boar taint is a complex phenotype, multiplying potential phenotyping methods, each with their own limitations, such as sensory analyses vs. quantifications of skatole and androstenone. 2169 Piétrain × Landrace boars were measured for three boar taint traits (sensory score, i.e., the reference trait, skatole and androstenone). These traits were evaluated in a MT evaluation. Then, their estimated breeding values (EBV), accompanied by their reliabilities (REL), were deregressed and obtained pseudo-phenotypes were evaluated in an ST evaluation. The used scalar deregression method had two limitations: a reduction in variance and a loss of reversibility. To solve this, an expansion factor and a reliability-alignment factor were iteratively applied in the pre-processing. The results are based on a comparison of EBV and REL from evaluations of the reference trait (i.e., in MT) and of the pseudo-phenotypes (i.e., in ST) according the two approaches: (1) standard deregression (DRP) and (2) adjusted deregression (ADJ_DRP). They showed that standard deregression indeed underestimates genetic variance, while adjusted deregression have (almost) identical distributions as in MT. The EBV(MT)~EBV(ADJ_DRP) regressions have slopes close to 1 and intercepts close to 0, confirming reversibility. REL remained stable. Therefore, pre-processing allows to effectively synthesise genetic information into single reference pseudo-phenotype while preserving essential genetic signal. The proposed method provides an extended reversible system adjusting deregression and generates pseudo-phenotypes compatible for subsequent use in routine genetic evaluation. Potential use includes the integration of pseudo-phenotypes into carcass evaluations in the framework of the Walloon Genetic Evaluation System for Piétrain boars. More theoretical studies are ongoing, as different partial data configurations including missing records for the reference trait, the application of higher-dimensional datasets and its transfer into a genomic context.

Selection for birth weight uniformity and its effects on subsequent weights in SRC Iberian pigs

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Sánchez Romero Carvajal (SRC) company is implementing a breeding selection scheme focused on the Iberian purebred animals, and its production system in open-air includes a finish-fattening period known as montanera based on an ad libitum intake of acorns and grass. This population has been selected for maternal traits since 2011 combining two traits: piglets born alive and total litter weight at 21 days, being the weight for each trait 80% and 20% respectively since 2019. Furthermore, the selection program for this population prioritized the genetic improvement of meat quality traits. Homogeneity in the weights at different steps in the breeding program is economically important and advantageous in farm management. Selection for birth weight (BW) uniformity has become an interesting selection criterion to be included in pig breeding programs. Within-litter variation of BW is of great importance in breeding practice for animal survival. In SRC breeding programs weights at different ages are collected. They routinely collect a preweaning weight around 21 days (PW), and since 2022 they have implemented the BW data collection and the weight around 90 days of age (AW). The objective of this study was to evaluate the impact of selection of BW uniformity in subsequent weights in the SRC population. Data contained 12,589 records of BW and PW and 1,589 of AW from two linked farms recorded between 2022 and 2024. The pedigree file contained 2,827 individuals. Univariate and multivariate heteroscedastic models were applied including age of weight for PW and AW, sex (2 levels), parity number (12 levels), litter size (13 levels), batch (23 levels) and sow feeding system (2 levels, for BW and PW) as fixed effects. The litter effect (for BW and PW) beside the maternal genetic effect were included as random effects. Same effects were included for the mean trait and its variability. The analysis was performed using ASReml 4.2. The maternal genetic variance affecting the environmental variability was 0.075 (0.024) for BW and 0.064 (0.024) for PW in univariate models. The litter effect variance was 0.265 (0.025) and 0.336 (0.027) for BW and PW variability, respectively. The genetic correlation between the trait and its environmental variance was not different from zero for both traits indicating the possibility of selecting for the mean traits and their variability independently. This is the opposite of what is seen in other commercial pig populations where this correlation is moderate and positive. This may be because the population has not been subjected to strong selection pressure to increase litter size, unlike the commercial populations. In the multivariate analysis, the genetic correlation between BW and PW environmental variabilities was 0.93 (0.05). AW trait seems not to be impacted by the environmental maternal genetic component. Applying BW uniformity to this population seems favourable for PW. Even though more data seems necessary for such complex models, other traits or other models should be essayed to deal with uniformity on latter weights after weaning.
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Potential to increase additive genetic variance in pigs by changing crossover count

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The goal of animal breeding is to select the best animals to reproduce to create genetic gain for multiple breeding goal traits in future generations. The rate of genetic gain increases when additive genetic variance is higher. One suggested way to increase genetic variance is by changing the number of meiotic crossovers (CO) that individuals transmit. CO allow homologous chromosomes to exchange DNA, generating novel allele combinations, which can release additive genetic variance. Hence, this study aimed to estimate additive genetic variance for breeding goal traits for different groups of individuals based on CO count in a sow pig line that was mainly selected for reproductive traits. We used 72,456 sire–offspring pairs with CO count observations, and observed CO count ranged between 5 and 34. We split the data in five bins based on the CO count and used bivariate sire mixed models to estimate the sire genetic variance in each of the bins. The bins were approximately of equal size and the CO ranges in each bin were: 5-14; 15-16; 17-18; 19-20 and 20-34 CO. Overall, results showed that sire additive genetic variance was not constant across bins, suggesting that CO may influence additive genetic variance for these traits. For the traits number of total born and number of born alive, the additive variance was maximized for intermediate CO ranges (~15-20). For number of stillborn piglets, additive variance was higher in lower CO counts. These results suggest that CO count might affect additive genetic variance between traits, and that CO ranges that could maximize additive genetic variance differs across breeding goal traits.

Heritability and Genetic Correlations of Crossover Count in Two Commercial Pig Lines

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The aim of animal breeding is to select the genetically best animals in the current generation to achieve genetic gain in future generations regarding a breeding goal. This genetic gain increases with a greater genetic variance. Crossover count (CC) is currently not directly selected for in animal breeding programs, but it might be beneficial to do so. Crossing-over between homologous chromosomes during meiosis generates variation in gametes, and can create more variable offspring. Crossovers can break linkage between alleles, resulting in new possible allele combinations in gametes and potentially increase additive genetic variance. It is therefore expected that selection for a higher crossover count can increase the genetic variance of other breeding goal traits, thereby resulting in a higher genetic gain. The number of crossovers is found to be variable between individuals, sexes, and species, and this variation is at least partly heritable which makes it feasible to change CC with selection. But before a new trait can be added to the breeding goal, it is important to know if there are correlations to other breeding goal traits. For CC, there are indications that its variation is directly associated with variation in fertility, because the formation of crossovers is a necessary step for successful completion of meiosis. In addition, variation in CC may be indirectly correlated to other breeding goal traits. Therefore, the main objective of this study is to estimate the heritability of CC and its genetic correlations with important breeding goal traits in two pig lines: a sow line and a boar line. Both lines consisted of >100,000 animals genotyped for ~20,000 SNP markers. The sow line was mainly selected for reproduction traits, and the boar line mainly for production traits. Phenotypic records are available on a number of breeding goal traits, including fertility, growth/feed efficiency traits, and the number of teats. The genotypes were phased to determine the number and locations of crossovers using LINKPHASE3. The autosomal CC was determined for 91,371 parent-offspring pairs in the dam line, and 150,228 parent-offspring pairs in the sire line. The first results showed that CC of the sow line was on average 23.9 for females and 18.1 for males. For the boar line, this was on average 22.4 for females and 17.4 in males. The higher CC in the sow line compared to the boar line is possibly an effect of selection on fertility traits. We expect to present the heritabilities of CC and their correlations to important breeding goal traits at the time of the conference, which will provide insight in the possibility of targeted selection on CC in the breeding goal, and whether CC may already be implicitly selected for, as a correlated response to the current breeding goal.

Methods for joint genetic prediction of multiple ordinal categorical and continuous traits

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The landscape of animal breeding is rapidly evolving. Data on health, disease, welfare, and fertility, which are key fitness traits for sustainable production, have been extensively collected. The categorical nature of these traits violates the normality assumptions of traditional linear mixed models, requiring threshold or threshold-linear models. These models can be fitted either via Gibbs sampling or by maximizing the posterior density (maximum a posteriori, MAP). While Gibbs sampling is flexible and can handle multiple-trait models with several categorical and continuous traits, its computational and memory demands, and the need to evaluate whether the Markov chain converged limit its applicability in genetic evaluations. MAP methods are computationally efficient and suitable for large-scale datasets. However, existing MAP methods are restricted to a single categorical trait and many continuous ones, making them insufficient for the new generation of data. The lack of proper methods to fit threshold-linear models to real-life applications has persisted for two to three decades. Failing to model categorical traits accurately can lead to reduced genetic gain and deterioration of the traits over time. This study aimed to develop the first theoretically sound and computationally efficient framework for jointly analyzing multiple categorical and linear traits, specifically multi-trait threshold-linear models, under BLUP and single-step genomic BLUP (ssGBLUP). The proposed methods are based on Newton-Raphson and Expectation-Maximization schemes. Using a simulated dataset and treating Gibbs sampling as the benchmark, we show that the breeding values estimated by our methods agree nearly perfectly with those obtained from Gibbs sampling, while greatly reducing computational cost and allowing scalability to very large datasets. As expected, the Newton-Raphson iteration outperformed the Expectation-Maximization algorithm in terms of computational time. Our results demonstrate that routine genetic evaluations incorporating multiple categorical traits are now feasible using the presented methodology. Solving this decades-old enigma enables accurate, large-scale genetic and genomic predictions of key categorical fitness traits under multi-trait models.

xyBnG.jl: A Julia package for scalable, GPU-accelerated stochastic simulation of complex national livestock breeding schemes

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Stochastic simulation of national livestock breeding programmes is a vital tool for evaluating the integration of novel traits, such as enteric methane emission (CH4), and optimizing complex multi-trait breeding objectives before field implementation. However, existing simulation tools often face computational bottlenecks in scale, speed, and memory when attempting to fully mimic national schemes that feature tens of thousands of animals, genomic selection, and intricate mating structures over multiple generations. We present xyBnG.jl, an open-source Julia package designed for the large-scale, full-complexity stochastic simulation of livestock breeding programmes. The software overcomes traditional constraints through advanced memory management and parallelization. It features a flexible BitArray-based genotype storage system capable of holding over 4.5 million individuals with 60K SNPs entirely in 64 GiB of RAM, drastically minimizing disk I/O. Furthermore, it incorporates CUDA-accelerated gene dropping, utilizing GPU power to exponentially speed up the processing of massive offspring populations. Analytically, xyBnG.jl seamlessly integrates Fisher-Wright historical population modeling, multi-trait maternal-effect PBLUP, and single-trait/multi-trait SNPBLUP for genomic prediction. To demonstrate its capabilities, we developed a digital twin of the Norwegian White Sheep (NWS) breeding scheme, accurately replicating its 12-trait Total Merit Index (incorporating growth, fleece, carcass, maternal, and CH4 traits), genomic selection pipeline, and complex ram circle mating system. We evaluated ten scenarios varying the selection weights for CH4 (0%, 2%, 5%, 10%) and the annual number of ewe CH4 phenotypes (0, 2K, 4K, 6K) added to the genomic prediction. The model simulated 12 years (5.5 generations) with 20 replicates, successfully generating and evaluating 120,000 lambs annually under realistic breeding conditions. The WCGALP simulation results verified by xyBnG.jl proved that incorporating CH4 into the national sheep breeding objective effectively reduces methane emission corrected for live weight, whilst simultaneously improving carcass and fleece weight. The most intensive scenario (10% weight, 6K phenotypes/year) achieved an emission reduction of 0.032 g CH4/hour per ewe over 12 years (0.37 genetic standard deviations). Larger selection weights and phenotyping intensity synergistically improved selection efficiency, but the benefits of additional phenotypes were constrained if the index weight for CH4 was insufficient (<5%). xyBnG.jl provides an efficient, reproducible, and highly scalable framework for simulating sophisticated national livestock breeding programmes. Its application to the NWS demonstrates that breeding strategies for reduced CH4 and improved production can be harmonized, underlining the necessity of strategic phenotyping investments. The package is freely available to the community to facilitate complex breeding programme analyses.

EVOGEN: A novel tool for large-scale simulation and optimization of breeding programs

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Animal breeding is a complex task as many factors such as prediction models, and the structure of breeding programs can influence the long-term genetic gains of focal traits. At every iteration it involves a sophisticated methodological toolchain that may include the methods of phenotypic and genomic evaluation. Therefore, simulation tools that are flexible while remaining user-friendly are useful for predicting responses to selection and for adapting breeding plans to achieve defined breeding objectives. EVOGEN software is a novel computational tool allowing in silico studies of general evolutionary processes (changes in natural populations, artificial selection/breeding, evolutionary computations) on population and individual levels. The software can be used for simulation, design, and optimization of a variety of selective breeding schemes of different levels of complexity. The software can simulate crossbreeding between any number of populations and traces genetic changes in these populations under different scenarios. It allows flexibility in simulation various population structures and accounts for different genetic models (with additive, dominance and environmental effects). It can be used to test different phenotyping and/or genotyping strategies, estimate breeding values, evaluate different selection and mating strategies, and generate genetic data to test statistical tools. EVOGEN differs from other simulation tools mostly by the implemented design principles, software interfaces, and the targeting environment while keeping similar levels of functionality and implemented features. EVOGEN is programmed in C++ and compiled into a shared library, which is interfaced to Python. Therefore, it allows scripting and interactive simulation in external environment to incorporate any other external tools to perform genomic evaluations, selection and mating. The software is designed for simulation of large-scale breeding schemes with many iterations, individuals, and genotypes. The software is well suited for a dedicated Linux based high performance computational environments. The software's design provides convenient interfaces that allow a user-defined low-level construction of simulation scenarios of different degree of dualization and complexity. Among others features, the most unique are a multiple-allelic genomic architecture and possibility of use the same interfaces for both tasks: design/simulation of breeding schemes and solving optimization problems using evolutionary computations, with genetic algorithms approach.

Beekeepers' preferences for breeding goals: how can the results from a French on-line survey feed the design of relevant breeding programs?

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Honey bee breeding initiatives are emerging worldwide. France is seeing the emergence of diverse small-scale breeding groups. Researchers are involved to support some of those bottom-up setups. The motivations of these beekeepers, the sizes, orientation and functioning of their production systems are highly diverse. Yet, little is known about what these diverse beekeepers would envision as fitting breeding goals in this context of relative novelty of bee breeding endeavours. In complementarity to comprehensive approaches of beekeepers' breeding practices, which often rest on context specific case studies, large scale inquiries are a useful way to get data on the diversity of breeding goals considered by beekeepers at a larger scale. However, relevant outputs of such large-scale surveys to support breeding programs design seemed lacking in honeybees. To fill this gap, an online survey was launched during most of 2023. We obtained about 250 complete answers, mostly from professionals. Answers gathered basic information about their beekeeping profile, including their main production and services offered, their bees' genetic background, their operation size, and more. Beekeepers were then asked to rate the importance they attributed to including certain traits in a breeding goal, choosing from a list of twenty possible traits and a three-levels importance scale: essential, useful, or useless. For useful or essential traits, they further were asked to indicate whether they wished to genetically see these traits improve, or only be maintained at their current level. Our findings revealed a strong inclination toward multi-trait breeding goals, with a median of 13 traits deemed as useful or essential. Such a result confirms the key role of multicriteria assessment processes in the design of relevant breeding programs. Honey yield, disease resistance, swarming tendency, gentleness, and summer feed autonomy (no emergency feeding required), stood out as the traits attributed the highest importance, with nearly 90% of beekeepers considering these traits at least useful.However, in a perspective of feasibility, including in terms of achievable genetic improvement, it is also key to know the nature of changes expected for those traits. In this survey. Around 40% or more of beekeepers stated they would prefer to see these traits maintained at their current level, as opposed to directionally improving them. A clear exception to this was disease resistance, with 75% of beekeepers wanting to directionally improve it.Regarding how beekeepers' profile shaped these preferences, the most significant characteristic was the genetic background of bees they kept, while other beekeeping profile characteristics only appeared to have marginal effects.Moreover, these results highlight several understudied traits, such as summer and winter feed autonomy, winter diapause, and longevity, that are regarded as at least useful in a breeding goal by over 70% of beekeepers, and for which nothing or only little is known about their measurement feasibility and potential to respond to selection. Consequently, such results are also useful to guide future research to identify and evaluate possible selection criteria for these traits.

Deriving cow methane traits and their genetic relationships with feed intake, energy balance and nitrogen utilisation efficiency

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Modern dairy cattle breeding goals suggest consideration of trait categories reflecting methane emissions, energy balance (EB) and nitrogen utilisation efficiency (NUE). However, detailed direct trait recording implies tremendous logistics and financial costs. Furthermore, for the development of an overall breeding goal for resource efficiency, the availability of genetic parameters and genetic correlations among all traits from all trait categories is imperative. EB and NUE indicators from routine monthly milk recording of 1107 German Holstein cows were energy corrected milk (ECM), fat-to-protein-ratio (FPR) and milk urea nitrogen (MUN). Based on calibration and prediction equations from milk mid-infrared (MIR) spectroscopy, NUE and EB traits were N-loss as the difference from nitrogen intake and nitrogen output in milk, NUE as their ratio, residual feed intake (RFI), and EB as energy intake minus the sum of milk energy and maintenance energy requirements. Methane traits were derived considering patterns of CH4 respiration and eructation from laser methane detector measurements and applying developed data analysis protocols. The generated methane traits included the arithmetic means (AllMean, RespMean, ErucMean) and the respective sums of emissions per minute (AllSum, RespSum, ErucSum). In a sub-sample of four research herds, the microbiome composition was characterized based on faeces analyses considering 237 faecal samples. 16S rRNA sequencing was performed to determine bacterial and archaeal community composition. Nanopore sequencing was applied to characterize the community of anaerobic fungi. Animal models were applied to estimate variance components and heritabilities for all "conventional" and MIR-based EB and NUE traits, and for all methane traits. Genetic correlations for all trait combinations were approximated based on estimated breeding value correlations of 43 sires with at least 10 daughters and corrections of breeding value accuracies. In enhanced mixed models, we defined the "milk traits" as explanatory variables, to infer their effects on all methane traits. Phenotypic association analyses indicated a significant effect of MUN on methane emissions, with significantly lower least-squares means for all methane traits of cows allocated to MUN classes < 150 ppm. FPR alterations as indicator for EB displayed only minor effects on CH4 traits, and respective genetic correlations were close to zero. For all CH4 traits, additive-genetic variation was identified, but the large residual component contributed to small heritabilities in the range from 0.001 to 0.02. Moderate heritabilities were estimated for the MIR-based traits with 0.28 for RFI, 0.24 for EB, 0.31 for N-loss and 0.13 for NUE. Genetic correlations between CH4 traits with EB and NUE indicators from routine milk recording (MUN, FPR, ECM) were close to zero, but stronger with the derived MIR traits, especially with N-loss in the range from 0.33-0.55. Consequently, CH4 emissions, EB and NUE displayed differing genetic mechanisms, indicating the need to include these trait categories separately into an overall breeding goal. CH4 emissions were weak indicators for EB and NUE, and vice versa, suggesting the implementation of detailed recording schemes. Microbiome-derived traits (relative abundance of key taxa, diversity indices, microbial community profiles) were phenotypically correlated with CH4, EB and NUE traits, suggesting the predominant role of the rumen microbiome composition regarding methane emissions and feed efficiency.

Statistical and genetic analysis of udder conformation traits recorded by automatic milking systems in Fleckvieh cows

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Udder conformation traits play a critical role in functionality and health of dairy cattle. Recent advances in automatic milking systems (AMS) have enabled precise, high-frequency recording of udder traits, providing valuable data for genetic analysis. Estimating genetic parameters of udder conformation traits allows breeders to ameliorate udder morphology genetically, ultimately improving functionality in both AMS and conventional milking systems, longevity and animal welfare. The present study aims to estimate the heritabilities and genetic correlations of udder conformation traits derived from AMS and classifier evaluations. This study utilised a comprehensive dataset comprising 3,006,575 observations of 78,458 Bavarian Fleckvieh cows across three parities, recorded over the period from 2019 to 2025 by Lely AMS. Data preparation and statistical analysis were performed using the SAS programme. The genetic parameters were estimated using the BLUPF90+ software. Five udder conformation traits were investigated in this study: front teat distance (FTD, mm), rear teat distance (RTD, mm), udder depth (UD, mm), udder balance (UB, mm), and distance front rear (DFR, mm). The descriptive statistical analysis of the AMS data is based on the exclusion of data outside the 1% and 99% quantile limits, in order to avoid extreme observations. The descriptive statistics of the conformation traits showed the following average values for the first three parities (P): FTD (P1=175; P2=178; P3=182), RTD (P1=91; P2=94; P3=98), UD (P1=587; P2=536; P3=501), UB (P1= 4.0; P2=-1.7; P3=-5.2), and DFR (P1=111; P2=128; P3=136). The results of analytical statistics revealed significant differences in least squares means based on 1–350 days in milk among parities in all five traits. The heritability was estimated solely for parity one, since classifier scorings were available only for first calving cows for the traits front and rear teat placement and udder depth. The estimated heritabilities of AMS conformation traits, including FTD, RTD, UD, UB, and DFR, were 0.54, 0.42, 0.47, 0.38, and 0.58, respectively. The phenotypic and genetic correlations between the FTD and front teat placement were negative (rp=-0.60; rg=-0.84). The negative correlations are plausible with regard to opposite definitions of AMS and classifier teat traits and consistent, when considering both RTD and rear teat placement (rp=-0.66; rg=-0.94). Conversely, positive correlations were identified between UD and udder depth recorded by classifiers (rp=0.66; rg=0.92). The estimated heritabilities for classifier evaluations for traits front and rear teat placement and udder depth were 0.33, 0.26, and 0.39, respectively. In conclusion, the present study revealed a higher heritability for udder conformation traits derived from AMS and promising genetic correlations with classifier evaluations. These results suggest a potential advantage by including AMS-based udder conformation traits in genetic evaluations, which should be further investigated.

Development of a new recording scheme for calf-health related traits

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Calf health and consequently mortality has long been a cause for concern with respect to animal welfare. More recently, circular economy considerations and consumers' desire to reduce food waste have additionally led to more attention being paid to losses along the food production chain. Apart from stillbirth, and lately, rearing losses, traits related to calves, youngstock and replacement heifers have largely been neglected in dairy and dual-purpose cattle breeding. In the joint Austrian-German-Czech genetic evaluation, the vitality value (VIW), which includes any stock losses including stillbirths in the first 10 months for male and the first 15 months for female youngstock, has been published since 2016. The VIW's relative economic value in the total merit index (GZW) is 5% in Fleckvieh and 4% in Brown Swiss. To further strengthen calf-related traits in the breeding objective, additional traits for health and mortality are to be added to the VIW within the ENSURE (Data driven strategies to enhance sustainability, resilience, and resource efficiency of cattle farming) project. In terms of breeding, using various data sources in addition to the existing ones is beneficial for representing different facets of calf health and survival, and for achieving higher reliabilities in potential breeding values. One such source is information on why calves left the herd, were culled or died, in line with the recording of disposal reasons for cows. The latter are used for management purposes but also as indicator traits in several genetic evaluations, since they are widely available in large quantities at little or no additional cost. Thus, the first step was to elaborate reasons for disposal or culling of youngstock. The focus is on young calves of both sexes until day 30 post-partum, and on female calves (i.e. replacement heifers) until their first calving. Apart from the categories "sold for breeding" and "sold for fattening", 14 disposal reasons were defined. These include e.g. respiratory problems, diarrhoea, umbilical hernia or septicaemia which mostly occur in younger calves, but also reasons relevant in youngstock, such as fertility problems or spastic paresis. Farmers can now record the codes in an upgraded app, allowing them to document the reasons for the disposal of calves and youngstock. In 500 farms taking part in the ENSURE project, recordings are monitored and supplemented by performance recording personnel during routine inspections, which take place eight to eleven times per year. Additionally, the app has been updated to include observations of contracted tendons and the sucking reflex. These are each recorded by farmers in five categories within 24 hours of birth. Similar to the disposal reasons, monitoring is accomplished by performance recording personnel in the 500 project farms. In next steps, the potential extension of existing data on individual calf health (i.e., veterinarian diagnoses) by information that currently cannot be linked to individuals (e.g., slaughterhouse or rendering reports), will be evaluated.

Genome-Wide Association Study of Navel Length at Weaning in Brangus Cattle

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Navel length (in females) and prepuce length (in males) are phenotypes that have been used in genetic evaluations of beef cattle more frequently in the last decade. These morphological traits are closely associated with the adaptation and functionality of the animals. Animals with long navels or prepuces are more susceptible to lesions from contact with pasture, which is undesirable in pasture-based farming systems. The objective of this study was to identify genomic regions associated with navel/prepuce length evaluated at weaning age in Brangus cattle. The dataset was provided by Gensys Consultores Associados, and it included phenotypes recorded from 182,937 Brangus (i.e., 3/8 Brahman and 5/8 Angus) animals raised at Brazil (south, southeast and midwest), Paraguay, and Bolivia. A total of 715,130 animals were included in the pedigree file, of each 11,592 animals were genotyped using a SNP panel containing 93,150 SNPs. The single-step GBLUP (ssGBLUP) method was used to predicted animals genomic breeding values (GEBVs), which were then back solved to estimate the SNP effects in the genome-wide association study (GWAS). The general model used include contemporary group (concatenation of sex, year, and farm of birth) and season in which the trait was evaluated (i.e., spring or fall) as categorical fixed effects, age of the animal at the time of evaluation as covariates (linear and quadratic effects), and the random effect of animal. Categorical phenotypes were used in the evaluation, ranging from 1 (short) to 5 (long length navels/prepuce). The Gibbsf90+ program was used to estimate the GEBVs using a threshold model, and PostGSf90 was used to predict marker effects. False discovery rate (FDR) was used to account for multiple testing. To select α values producing an FDR lower than 0.05, the p-value rank position procedure was used. The GWAS resulted in 103 significant single-nucleotide polymorphisms (SNPs), at an empirical threshold P-value of 3.38×10^{-5} (FDR < 0.05). Those SNPs were located at 28-60Mb at BTA5, with the majority around of 40-55Mb (70%). This region contains the WIF1 gene (48.6-48.7 Mb), which is known to be related to navel length in cattle. In addition, other QTLs associated with milk protein production in dairy cattle and kidney fat weight in Brangus cattle were identified in the top region identified. Studies on navel length are still scarce in the literature, but our results point to an important genomic region possibly regulating this trait, reinforcing previous studies focused on other breeds.

Phenotyping strategies to enhance genetic gain for animal health and welfare in dairy cattle – from the past to future perspectives

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Sustainable cattle breeding requires the integration of new data sources and technologies to improve the resilience, efficiency and environmental performance of production systems. One of the expected main results of the ENSURE project is the revision, further development and optimisation of breeding strategies for Fleckvieh and Brown Swiss to increase sustainability and resilience. This includes novel phenotypes for traits related to resilience, reproduction and health derived from technological advances and new data sources; the evaluation of different phenotyping strategies; the development of a breeding goal that considers biological, economic, environmental and consumer aspects; and the optimisation of the entire breeding process. In genetic improvement programmes, traits can be broadly classified as either direct or indirect. Indirect traits, often referred to as indicator traits, are genetically correlated with the trait of interest, but are usually cheaper or easier to measure. This is particularly important for traits with low heritability, such as many health-related traits, for which large datasets are required to achieve reliable breeding values. However, direct traits are generally more efficient for achieving genetic progress when comparable data quantities are available. They are also essential for developing and validating indicator traits. Currently, phenotypes within breeding programmes for health and welfare are traditionally based on diagnoses from veterinarians and hoof trimmers, farmer observations and recordings within dairy herd improvement programmes, and laboratory results, with a growing interest in mid-infrared (MIR) predictors. Technological advances offer new possibilities for novel phenotypes. This presentation will discuss different approaches, the requirements and challenges of various traits, and strategies for genomic breeding programmes. It will focus on genetic improvement in terms of health and selected welfare traits in general, as well as different direct and indirect traits relating to claw health and lameness. Direct traits include diagnoses from veterinarians and claw trimmers. Other data sources include information on lameness and hind claw position, as well as changes in milking and behaviour (e.g. activity, eating and lying down). Novel technologies provide information to assist with management decisions. The presentation will present existing research results, as well as ongoing research within the ENSURE project.

An impact of including methane emission into the Polish Holstein-Friesian cattle breeding program

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The genetic progress in dairy cattle breeding depends on the balancing of multiple selection criteria. In the Polish Holstein-Friesian (PHF) population, the current selection index integrates production, conformation, and functional traits. However, the dynamic nature of the dairy industry – particularly the increasing demand for environmental sustainability – requires re-evaluation of trait weightings and the introduction of new selection objectives, such as methane emissions (CH4), to ensure long-term genetic gain. This study aimed to evaluate and compare several alternative scenarios of including CH4 trait into breeding objective and compare the effects with the current PHF breeding goal. We simulated the complex population structure of Polish dairy cattle, using the Modular Breeding Program Simulation (MoBPS) framework in R,. The simulation involved a multi-generational approach, where different selection strategies were tested by varying: 1) relative weights assigned to specific sub-indices (production vs. functional traits); 2) inclusion of methane emission as an additional trait; 3) intensity of selection within the sire and dam pathways, and the impact of genetic correlations between methane traits and performance traits. The performance of each scenario was assessed based on predicted genetic response, inbreeding rate, and overall environmental efficiency. The findings illustrate how reallocating selection pressure affects the long-term genetic correlation structure, showing that methane reduction can be achieved while maintaining stable genetic gains in production and functional traits. This comparative analysis provides objective selection criteria foundation for future modifications of the national selection index, ensuring that breeding goals remain aligned with both industry requirements and global climate targets. The European Union's Horizon Europe research and innovation program provided funds for this work under grant agreement no. 1059609 (Re-Livestock project).

Genetic background of Navel Length in Brangus Cattle

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Genetic background of Navel Length in Brangus Cattle Thaise Pinto de Melo1, Daniele Alves de Oliveira1, Saymon Rocha1, Sirlene Lazaro2, Hinayah Rojas de Oliveira3 1Federal University of Santa Maria, Departament of Animal Science, Santa Maria, Rio Grande do Sul, Brazil. 2University of Guelph, Departament of Animal Biosciences, Guelph, Ontario, Canada. 3Purdue University, Departament of Animal Sciences, West Lafayette, United States. Navel length has recently been considered as an important trait in genetic evaluations of beef cattle. Its importance lies in its relationship with animal welfare and the adaptability of cattle raised in pasture-based systems. Navel length is considered as a categorical trait, generally evaluated based on five classes. Animals with greater navel length tend to have more problems with inflammation in the navel and prepuce regions, and are therefore undesirable. Due to past selection pressures, animals in classes four and five (i.e., with longer navels) are often underrepresented. The objective of this study is to evaluate navel length phenotype as categorical (using either five, four, and three categories), and linear models, based on both BLUP and ssGBLUP methods, and to assess their impact on heritability estimates, accuracy, bias, and dispersion of breeding values. The database contained information from 182,937 animals, as well as pedigree information for 715,130 individuals. A total of 11,592 animals were genotyped using a SNP panel containing 93,150 SNPs. Of the 182,937 animals, 6,437 (3.52%) belonged to the phenotypic classes four or five, i.e., presented the longer navel phenotype. The BLUP and ssGBLUP models were tested using the BLUPF90 program family. The results showed that the variability for the results for evaluated parameters among the different scenarios was generally low. Among all, heritability was the parameter that varied the most, ranging from 0.52 to 0.38 in the models and scenarios tested. The scenarios using with five or four classes produced higher heritabilities and were closer to the literature, with low variation between BLUP and ssGBLUP. In the scenario using only three classes, there was a slight reduction in the heritabilities to 0.48 (BLUP), and a greater decreasing was observed when the trait was treated as linear (0.38 for BLUP). Overall, accuracies ranged from 0.35 to 0.38; bias ranged from 0.02 to 0.06, and dispersion ranged from 0.83 to 0.88. The small variation and little difference between scenarios can possibly be explained by the low number of animals in classes four and five, only 3.52% of the total number of phenotypes, and only 654 of these were in the validation population (younger animals with genotype). Therefore, the different methods of dealing with navel length in cattle did not have a major impact on the parameters evaluated, except for heritabilities.

Genome-Wide Association Study for Hair Coat Score in Brangus Cattle

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Hair coat score is a key adaptive trait for tropical and subtropical cattle breeds, influencing heat dissipation, ectoparasite resistance, and overall fitness in Brangus (Bos taurus × Bos indicus). Despite its economic importance, genomic regions underlying weaning coat score remain poorly characterized. This study aimed to perform a genome-wide association study in order to identify important genomic regions associated with hair coat score in Brangus cattle at weaning. The analysis used Natura-Gensys® Brangus data (from Brazil/Paraguay/Bolivia): 70,045 phenotypes of hair coat scores evaluated at weaning (1-3 scale, ~205 days), 11,592 genotyped animals for 93,150 SNP markers, 715,130 pedigree records. We used the single-step GBLUP (ssGBLUP) method in order to predict the genomic breeding values (GEBVs), which were then back solved to estimate the SNP effects in the genome-wide association study (GWAS). Contemporary groups were formed by concatenating information of year, farm, season and management group. BLUPF90 family programs were used to perform the analysis. Windows of 1 Mb were constructed and the percentage of variance explained by each window was evaluated, ordering the top windows that explained 10% of the total genetic variance together. A total of 36 windows collectively explained ~10% of the additive genetic variance. BTA9 showed the highest number of windows (four), whereas BTA4 concentrated three of the most informative windows, jointly explaining about 1.5% of the variance: BTA4_69 (69.93–70.90 Mb; 0.868%), BTA4_9 (9.40–10.39 Mb; 0.378%) and BTA4_10 (10.75–11.75 Mb; 0.216%), with BTA4_9 and BTA4_10 forming a consistent cluster at 9–11 Mb. BTA20_35 (35.83–36.77 Mb; 0.349%) covers the SLICK region, including SKP2 (38 Mb), SPEF2 (38.3 Mb), and nearby PRLR, AGXT2 and DNAJC21 (~39 Mb) – major players in hair coat and heat tolerance. Cumulative variance reached 3.72% in the top 10 windows. These results indicate that weaning coat score in Brangus has a complex, highly polygenic architecture, controlled by many loci of small effect rather than a single pronounced peak, with BTA4 emerging as a relevant hotspot for future fine-mapping and functional studies in the 9–11 Mb and 69–71 Mb regions.

Genomic evaluation of weaning coat score in Brangus cattle using BLUP and ssGBLUP

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Hair coat score at weaning is a key adaptive trait for thermoregulation and ectoparasite resistance in Brangus cattle maintained under tropical and subtropical heat stress. Short slick coats (score 1) enhance radiative heat loss while reducing tick harboring capacity, critical for this Bos indicus-influenced breed in low-latitude production systems. In genomic prediction, the single-step genomic best linear unbiased prediction (ssGBLUP) method allows the inclusion of information from genotyped and non-genotyped relatives in the analysis. This study aimed to perform BLUP and ssGBLUP genomic evaluation in commercial Brangus populations, estimating prediction accuracy, bias, and dispersion for both methods. The analysis used Natura-Gensys® Brangus data (from Brazil/Paraguay/Bolivia): 70,045 phenotypes of hair coat scores evaluated at weaning (1-3 scale, ~205 days), pedigree (n=715,130 animals), and genotypes from 11,592 animals. Contemporary groups were formed by concatenating information of year, farm, season and management group. BLUPF90 family programs were used to perform the analyses with fixed contemporary group and age-at-weaning covariate, plus random additive and residual effects. Both methods estimated moderate heritability (0.45) for weaning hair coat score. Forward validation (2020-2024 cohorts) used linear regression of full-dataset EBVs/GEBVs against deregressed breeding values from validation animals only, with ssGBLUP based on 6,846 genotyped animals and BLUP on 9,522 animals. The accuracies with ssGBLUP were higher than those from traditional BLUP, with accuracy of 0.40 and 0.35, dispersion of 0.80 and 0.81, and bias of −0.010 and −0.009 for ssGBLUP and BLUP, respectively. Despite the smaller genotyped validation set, ssGBLUP showed a 15% increase in accuracy with comparable dispersion (b_1≈0.80; mild under-dispersion typical without metafounders) and stable heritability, supporting its use for genomic selection of tropical adaptation traits at weaning in Brangus cattle.

Supporting dairy cows breeding practices using historical fertility data and MIR spectra : a deep learning approach

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Holicow is an Interreg North-West Europe (NWE) project dedicated to the development of a holistic herd management tool for dairy farmers. The project capitalizes on an extensive transnational database comprising more than 60 million milk recording samples and over 500 existing predictive models based on Mid-Infrared (MIR) spectroscopy. In addition, more than 19 million reproductive events (primarily inseminations, pregnancy diagnoses, and calvings) have been collected by the project partners. The primary objective of this work was to integrate MIR spectral data and derived predictions with fertility records in order to develop robust models capable of predicting key reproductive traits directly from routine milk recordings. A first major step consisted in identifying fertility traits that are both relevant for on-farm decision-making and available, either directly or indirectly, within the transnational database. The main traits investigated were pregnancy status, probability of success at the time of insemination, and probability of embryonic loss. Subsequently, predictive models were developed for these traits using either raw MIR spectra or MIR-based predictions as input variables. Both classical machine learning approaches and deep learning techniques were implemented and evaluated. Combined with existing methodologies for assessing milk production, milk processing characteristics, animal welfare, environmental impact, and heat stress, this approach contributes to the development of a comprehensive and collaborative decision-support tool accessible to farmers and advisors.

GEDA: A novel software for automated processing of sniffer-based gas emission measurements

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Accurate estimation of methane (CH_4) and carbon dioxide (CO_2) emissions is essential for genetic evaluation of environmental impact and supporting mitigation strategies. In this context, sniffer-based measurements play substantial role for upscaling number of records in a cost-effective manner. Sniffers integrated into Automatic Milking Systems (AMS), provide continuous gas concentration records; however, these data are often affected by sampling irregularities, sensor noise, time misalignment between data streams, and environmental drift. Therefore, the quality of such records is a well-known challenge and alongside emissions' phenotypes estimation is critical to address. This study introduces the GEDA software representing a unified framework for processing raw sniffer data into aligned, quality-controlled gas emission data and reliable emission phenotypes. The pipeline implementation is based on a stochastic model that represents noisy gas emission signals as a function of AMS data. This formulation enables the workflow that integrates data quality control, errors correction, synchronization of AMS and sniffers data following reliability assessment. The background concentrations are estimated through a weighted regression model following data corrections. Extreme outliers are identified and bounded according to parameter-based statistical criteria. Optional denoising is implemented using singular spectrum analysis. After visit-level filtering, emission phenotypes are calculated as mean concentrations [ppm] or emission intensities [ppm/min] at visit-level or daily-level. The quality of the phenotypes is evaluated using linear mixed models to estimate the intraclass correlation coefficient as a localized quality metric. A comprehensive case study based on four years data from 38 Danish commercial farms assessed the pipeline's efficiency and the quality of estimated phenotypes. In this analysis, records were filtered using thresholds of the local quality metric, and heritability and repeatability were subsequently estimated for each dataset. Heritability estimates for daily average phenotypes ranged from 0.13 – 0.16, while repeatability values were in the range of 0.32 – 0.41. The pipeline parameter sensitivity was assessed for sampling frequency, signal length, scaling, denoising, visit constraints, outlier filtering, and phenotype definition. Maximum repeatability was observed at a data sampling interval of 1 s. Repeatability was highest at a minimum visit duration of 2 min and decreased as the exclusion window increased. Among filtering parameters, the upper outlier bound had the largest effect on repeatability. Finally, visit-based intensity phenotypes provide more stable (to the changes in data sampling rates) repeatability estimations than daily or concentration-based alternative. On average, processing approximately 1 million records required 2.74 min using 6 CPU cores. Overall, GEDA provides a theoretically grounded framework for processing sniffer-based gas emission data under commercial farm conditions. By addressing data heterogeneity, noise, and misalignment, the pipeline enables the generation of aligned AMS and gas concentration datasets and emission phenotypes suitable for genetic evaluation and greenhouse gas research in dairy cattle.

Should she stay or should she go now? Ranking of cull cows based on Net Present Value implemented in Dairy Management Software

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Decisions regarding culling dairy cows are complex and many factors influence the decision. Farmers may aim to keep the most profitable cows, but profitability can be difficult to assess and are often made in a nonprogrammed fashion, relying partly on intuition. To support the day-to-day decision of which cows are candidates for culling, ranking of the cows based on the Net Present Value (NPV) has shown to be useful. The NPV estimates the discounted, future cash flows of a cow, including both revenues and costs. The objective of this project was to implement an NPV ranking of cows in the Danish Dairy Management Software, DMS, supplementing the existing data on performance with an estimation of each cow's future economic outcome. A dynamic, stochastic and mechanistic simulation model (the SimHerd Model) was used in a large simulation study of cash flows of different cow types. The SimHerd Model simulates a dairy herd in weekly steps; representing production, events and state of each individual cow in the herd. The simulated cow types were based on parity, lactation stage, milk yield, plus reproductive and health status. All cow types were simulated in different herd types characterised by milk yield, culling level and reproductive performance. Based on the simulated data, response surface models (RSMs) were developed to predict the NPV of individual cow type in the different herd types. In the Danish DMS, the cow level data on milk recordings, reproduction events and health registrations are used as input for the RSM models. As the RSM models are tailored to different herd types, The individual NPV is calculated after each monthly milk recording for all lactating cows with milk recordings >70 DIM. The herd and cow specific NPV are converted into values on a ranking index from -100 to +100. In January 2026, all dairy farmers with access to DMS were presented with the ranking index in a dashboard together with supplemental information regarding the cow's performance in the current lactation. Cows not eligible for culling, due to medicine use or near calving, are marked. The user can filter which group of cows are of interest. The dashboard has been well received with predominantly positive response to the new tool from the farmers. Ranking of culling candidates based on the NPV was successfully implemented in a dairy management software with national reach. Farmers find the add-on with the future economic value useful in combination with the historic data on the cows.

High-throughput video phenotyping of sow activity around farrowing and its association with piglet mortality

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Sow postural activity is an overall indicator of maternal behaviour and health status of sows, both of which have a strong influence on piglet survival. The aim of this study was to develop and apply a high-throughput video phenotyping approach to characterize sow activity around farrowing and to evaluate its association with piglet mortality. A total of 134 Large White sows kept in farrowing crates in the INRAE experimental facility were video recorded. The database included 221 farrowing events between April 2021 and April 2024. A YOLO convolutional neural network was trained for automated image classification to predict sow posture. The network was trained on diverse images to ensure high predictive accuracy. The algorithm achieved a global accuracy greater than 95% on all postures but one. The model identified eight postures that were predicted at one-second resolution: udder left/right, lateral left/right, sternal, sitting, kneeling and standing. Analyses focused on −3 days to +5 days relative to the onset of farrowing (birth of the first piglet). After filtering, 1,099,545 posture change events were used for analysis. Sow activity was characterised by posture duration, frequency of posture changes, and posture sequences using a sliding-window approach across periods before, during (6 hours after onset of farrowing), and after farrowing. Features were summarized using mean, SD, area under the curve, peak-to-mean ratio (PTM), and PTM_SD. Of the 35,161 generated activity traits, 27,506 were retained. On average, activity increased during the 24 h preceding farrowing, peaked shortly before onset, and declined immediately afterward. Mean posture duration decreased toward farrowing and increased post-partum. Inter-individual variability was observed in activity intensity and posture transitions across different time periods. To identify activity traits most strongly associated with mortality traits, we applied Elastic Net regression within a multi-response linear model using the glmnet package in R software. Piglet mortality traits comprised 23 continuous variables (e.g., stillborn (SB), crushed (CR), weak (WE), savaged (SA), killed (KI, i.e., CR + SA), number of piglets born alive, litter sizes at days 0 to+3), corrected for parity, batch, and total number born. Elastic Net selected 150 activity traits among which 19 key traits were significantly correlated with SB, CR, WE, and KI ($p < 0.01$). SB showed the strongest mean correlation ($|r| = 0.38$, 11 traits, mostly post-farrowing), followed by killed (0.26, 7 traits before and during farrowing), CR (0.20, 3 traits during farrowing), and WE (0.18, 1 trait during farrowing). Duration in kneeling posture before farrowing was associated with KI and CR. Increase in sequences of active postures during farrowing were positively associated with KI and CR and negatively with WE. The strongest association ($r = 0.62$) was between SB and mean frequency of short (<60 s) udder-left postures measured over 4-hour sliding windows, starting 6 hours after the onset of farrowing. These findings show that high-throughput video phenotyping can reveal moderate to strong associations between maternal activity patterns and piglet mortality. Automated, continuous, and standardized quantification of activity traits offers strong potential for precision monitoring, integrating sow activity into breeding strategies, and anticipating farrowing or health issues.

Seasonal adaptation is associated with systemic metabolic remodelling in heavy pigs: a molecular phenomic approach

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Seasonal thermal conditions represent a relevant environmental challenge for finishing pigs, potentially influencing metabolic regulation, physiological homeostasis, and performance outcomes at high slaughter weights. In heavy pigs (approx. 160 kg), environmental conditions may influence systemic metabolic status, with potential implications for physiological robustness and carcass characteristics, which may in turn affect the subsequent seasoning of ham. Molecular phenomic approaches, including plasma metabolomics, provides a valuable tool to characterise integrated physiological responses to environmental variation under field conditions. However, seasonal metabolic variation in heavy pigs has never been characterised using plasma metabolomics. The objective of this study was to characterise seasonal metabolic differences in performance-tested Italian Large White heavy pigs at slaughter using untargeted plasma metabolomics and to evaluate systemic metabolic adjustments associated with pre-slaughter temperature conditions. A total of 193 Italian Large White pigs were included in two seasonal groups: summer, collected across four slaughtering days (n = 87), and winter, collected across three slaughtering days (n = 106), balanced between males and females. Pre-slaughter thermal exposure differed markedly between seasons, with higher 10-day average minimum and maximum temperatures in summer (average min 20.92 °C; max 33.90 °C) compared to winter (average min -3.77 °C; max 3.2 °C). Plasma was collected at slaughter, which was performed in the early morning. Untargeted metabolomics detected 670 metabolites. After quality control, exclusion of xenobiotics, and removal of metabolites with >25% missing values, 580 metabolites were retained for downstream analysis, which included imputation using Multivariate Imputation by Chained Equations. Seasonal comparisons were performed using the Wilcoxon rank-sum test and linear regression. Only metabolites with a Bonferroni-corrected p-value < 0.01 in both analyses were considered significant. Enrichment analysis of significant metabolites was performed using MetaboAnalyst, considering the Relational Database of Metabolomic Pathways and requiring at least three entries per cluster. A total of 193 and 187 metabolites were detected by the Wilcoxon and linear regression analyses respectively, with an overlap of 177 metabolites. Enrichment analysis (RaMP DB) indicated that discriminant plasma metabolites clustered in pathways related to membrane transport, lipid metabolism/signaling, amino acid and branched-chain amino acid catabolism, and nucleotide/pyrimidine metabolism, with additional enrichment for mitochondrial biogenesis (FDR < 0.01). These findings indicate that seasonal temperature variation is associated with coordinated systemic metabolic remodelling in heavy pigs, detectable at slaughter, and identify potential plasma biomarkers of thermal adaptation under field conditions. Research funded by the European Union – NextGenerationEU under the National Recovery and Resilience Plan (PNRR) – FEEDTHEPIG, proposal code P2022FZMJ9 – CUP J53D23018310001 and the European Union's Horizon Europe research and innovation programme under the grant agreement No. 101059609 (Re-Livestock).

Comparison of classification models to estimate lifetime productivity of Murciano-Granadina goats from first-lactation records

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Lifetime productivity is a key driver of the economic and environmental sustainability of dairy goat systems. However, culling and replacement decisions are still largely based on current milk yield without considering lifetime production, although an increased longevity may improve herd efficiency. Milk recording programmes routinely provide detailed individual data, creating an opportunity to predict lifetime performance at an early stage and support more informed farmer decisions. The aim of this study was to compare classification modelling approaches to predict individual lifetime performance indicators in Murciano-Granadina (MG) goats using information available at the end of the first lactation. Data were obtained from 76 MG dairy goat herds and included 24,459 goats with complete productive life records. The database integrated three data sources: (i) milk recording data (e.g., 270-days milk yield and composition, lactation length, etc.), (ii) morphological evaluations (e.g., general structure, udder system, etc.), and (iii) lifetime performance of the dams. Target variables were lifetime milk yield, total days in milk (DIM), longevity and total number of lactations. Each target trait was discretised into four ordered groups using percentile-based cut-offs (P25, P50 and P75). After data cleaning and standardisation, models were trained using different combinations of predictor sets, all of them including milk-recording variables. Four classification approaches were evaluated: (1) logistic regression (LR), a well-known linear model for nominal classification; (2) ridge regression (RR),), a regression-based approach trained on continuous targets, whose continuous predictions are subsequently mapped to classes; (3) ordinal ridge regression (ORR), a naïve ordinal classifier trained on categorical data that produces continuous outputs, which are then converted into class labels; and (4) a logistic model with all-threshold variant (AT), specifically designed for ordinal outcomes. Model performance was assessed as classification accuracy (ACC) and mean absolute error (MAE) for each data-model combination. The former evaluates the proportion of correctly classified goats, without considering the magnitude of the errors, whereas the latter measures the average number of categories by which the prediction deviates from the target category. Combining milk-recording and morphological data yielded the lowest MAE, whereas models based on milk-recording data alone showed the highest error. AT achieved the lowest MAE for lifetime milk yield (0.74), longevity (0.81) and number of lactations (0.93), with only minor reductions in accuracy compared with the maximum observed values (−0.04, −0.05 and −0.09, respectively). In contrast, RR provided the best results for total DIM (MAE=0.65; ACC=0.42, the highest observed). Overall, modelling lifetime traits as an ordinal classification problem reduced MAE with only a modest loss of accuracy. This is particularly relevant for decisions at farm level, where avoiding misclassification of individual animals is critical. These results support the use of routinely collected lactation data, complemented with morphological and maternal information, as an early decision-support tool to improve farmer decision making.

Is gut microbiome information to be considered as part of the animal phenomic realm?

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Animal the gut microbiota (GM) has been proposed for its unique potential to describe and interpret complex host phenotypes, reflecting genetic and environmental influences and their interplay. Lastly, the GM has been proposed as the ultimate tool to explain animal resilience, for its nature of representing a dynamic and responsive ecosystem, defined and determined in tight relationship with the animal's physiology, So to give a broad definition encompassing both ruminants' and monogastrics' physiology, GM could be defined as the complex ecosystem of symbiont organisms that inhabits the gastro-intestinal tract. At the same time, animal phenomics (AP) has been developed as its own field. AP can be defined as the ensemble of technologies that can be used to gather, organize and manage data relative to animal phenotypes. AP is receiving strong attention from both the scientific community and the industry, for the unprecedented opportunities it can create in monitoring and controlling animal production system. Economic profit, animal welfare and environmental impact can all be improved thanks to AP. With that being said, can GM be considered as a form of AP? Several studies have attempted to used GM in this sense, which can all be brought to our attention. However, there is probably a lack of conceptual foundation for its proper use as an AP feature. While AP technologies are designed to capture animal variability, GM is an intrinsic characteristics of animal variability. GM is a feature that determines animal variability. This talk will provide a dialectic analysis of why GM can or cannot be used as an AP feature. This will encompass animal management and breeding. As a likely conclusion, GM can't probably be declared as the most appropriate source of information for use as AP, but some applications in animal breeding appear promising.

Exploring European Family Farmers' Attitudes Towards Big Data Tools

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Exploring European Family Farmers' Attitudes Towards Big Data Tools Family farms account for the vast majority of farms within Europe (more than 9 out of 10 farms) and their resilience and survival are key to European food security and resilience of rural communities. Therefore, understanding from a bottom-up perspective their needs and expectations from on-farm technologies that could aid in their resilience and survival is critical. The agricultural sector is experiencing its fourth revolution, Agriculture 4.0., – characterized by the use of smart farming tools such as the Internet of Things (IoT), robotics, sensors, machine learning, big data, cloud computing, etc. In recent years, the use of big data tools in farming have increased globally, across all types of farms, and these tools are expected to have a major impact within agriculture and possibly change the way farming is done today. Data from digital tools, like big data tools, both start and end at the farm. From data collection stemming from the farm, it is processed and used in a vast multitude of ways before returning back in various formats (such as data analytics for assisted decision making, livestock health and welfare tracking) to benefit the farm. For example, as EFFAB (the European Forum of Farm Animal Breeders) members wide use of digital tools show, data is utilized to connect genotype to phenotype (G2P), develop and improve sustainable breeding, and much more. This is why collecting this herd and animal level data from farms is important for the whole livestock sector in order to improve resilience and sustainability. As these novel technologies are being developed, employing a co-design process is crucial to ensure fair and practical tools are being created for European family farmers. Additionally, concerns have been raised that these tools could increase the power divide within farming, and in turn be a disadvantage for smaller farms rather than a beneficial addition enhancing sustainability and resilience of their farms. Therefore, this study aimed to inform the development of tools that actively involve family farmers in the creation process by exploring the research questions: What are family farmers' attitudes towards participating in the co-design for big data tools? What skills and support are needed by family farmers to adopt big data tools? What are family farmers' needs and expectations from big data tools? What are family farmers' concerns and perceived benefits towards current and future use of big data tools? This study used a mixed methods approach, with semi-structured interviews employed to explore research questions 1 and 2, and an online survey questionnaire addressed questions 3 and 4. Overall, this study's findings suggest that for farmers who have already adopted big data tools, the tools are having a positive and significant impact on their day-to-day farm work and decision making. As these tools are further developed, it is crucial to engage farmers in the co-design process to address their concerns, needs, and wants (including support and training needs pre- and post-adoption) – which will aid to increase big data tools' usage and uptake. Ensuring fair and just tools, and access to adoption is key to ensure family farms' resilience and survival.

Use of new digital and "omics" technologies data in animal breeding: opportunities and issues

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Animal breeding has long been based on the use of large amounts of data, either phenotypes, pedigrees or genomic data, most often specifically collected for breeding purposes. The explosion in recent years of high-throughput data collected automatically on animals (weights or volumes, data from robots, sensors, accelerometers, cameras, microphones, etc.) and their rearing environment, from animals' tissue samples (genetic markers or sequences, transcriptomes, epigenomes, microbiomes, proteomes, metabolomes, etc.), in vitro (cell cultures, organoids, etc.), and in silico (data generated by imputation, modelling, etc.) offers numerous opportunities for animal breeding. The primary use of some of these data is not breeding, and its use in breeding raises a number of challenges and difficulties. This article presents different potential uses use of these new data to characterise multi-performance, adaptation to climate change and to expected changes in livestock systems, as well as some problems and issues that may arise (data quality, standardisation, interoperability, data ownership, etc.). Authors received financial support from the European Union's Horizon Europe Coordination and Support Action under grant agreement no. 101134866, (project Digi4Live, https://digi4live.eu/) and from French National Rechearch agency,France 2030 program under the reference ANR-22-PEAE-0013, https://www.pepr-agroeconum.fr/.

Economics of smart livestock farming technologies – Current costs and benefits and the need for more harmonised estimation approaches

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Sensor technologies, such as milking robots or accelerometers are widely used on dairy farms, especially to monitor animal health, feeding and milk-related aspects. Although sensor technologies are common, information about the economics of their application is scattered. While existing studies illustrate cases where the use of digital technologies in livestock systems yields economic benefits, comprehensive assessments that capture overarching trends, the consistency of reported effects, and the broader economic implications for both researchers and practitioners remain absent. The aim of this study was to synthesize economic implications of sensor technologies in dairy farming. A literature review on sensors in dairy farming that was conducted according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines. The Scopus and Web of Science databases were searched using economic, sensor and animal type as search terms. From 1 355 identified publications, only 22 studies provided content that was in accordance with the criteria. In total, 11 studies focused on economic efficiency of automatic milking systems (AMS) for milk production monitoring, seven looked at various sensors (accelerometer, camera and intervaginal) for estrus detection, three examined information of sensor application in lameness detection and one focused on the economics of using sensors for body condition score and animal identification monitoring. The highest variation in the net profit of investment was observed for milk production monitoring with automatic milking systems, followed by estrus detection. The break-even costs of various sensors used for lameness detection varied between €6 and €53, on average. The profitability of sensors is linked with the severity of issues that information obtained by sensors could mitigate, the accuracy of sensor data to measure traits and opportunities to respond to the situation. Comparison between various economic results is hampered by different costs and methods used. To provide more robust evidence on economic impacts of digital and data-integrated solutions, a systematic data collection approach is proposed. In this approach, indicators will be determined using a literature review and consultations with researchers and stakeholders. Data collection instruments will be developed and validated by using a co-creation approach. Finally, we elaborate the importance of a harmonised approach that ensures comparability across studies. Authors received financial support from the European Union's Horizon Europe Coordination and Support Action under grant agreement no. 101134866, (project Digi4Live, https://digi4live.eu/).

Stakeholder Role Differences in the Use of Precision Livestock Farming Technologies in Europe

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Precision livestock farming (PLF) technologies are increasingly recognised as part of broader socio-technical systems linking livestock monitoring technologies, data infrastructures, and actors across the livestock value chain. Stakeholders occupy different structural positions in these systems, which may shape technology deployment, performance perceptions, and data governance. Within the Horizon Europe project Digi4Live, we surveyed 92 stakeholders across EU member states (farmers 33.7%; non-farmers 66.3%) to examine role-based differences in current PLF context. Technology type selection was associated with both stakeholders' roles ($p<0.001$) and livestock species ($p=0.002$). On-animal sensors dominated overall and were reported almost exclusively by farmers, while non-farmers described more diversified portfolios including off-animal and integrated systems. Across species, on-animal and integrated systems were more common in cattle production, whereas off-animal systems were more prevalent in pig production. Activity and motion (n=63, 69.2%) were the most frequently reported monitoring indicators across roles ($p=0.15$), while primary objectives differed ($p=0.01$), with farmers prioritising reproduction management and non-farmers emphasising health monitoring. Respondents reported broadly positive evaluations of technologies performance: data accuracy satisfaction ($M = 3.75 \pm 0.87$), perceived effectiveness ($M = 3.60 \pm 1.05$), and validation realism ($M = 3.61 \pm 0.91$) were all significantly above neutral level (1=poor, 3=neutral, 5=excellent, all $p<0.001$), with no significant role differences (all $p > 0.05$). In contrast, governance perceptions diverged significantly between roles. Farmers more frequently reported being unaware of how technologies were validated (n =21, 67.7%; $p < 0.001$). Differences also emerged in data-sharing preferences ($p=0.026$) and perceived barriers ($p = 0.031$): farmers were more likely to share data for research purposes (n = 15, 48.4%) and more often reported no significant barriers to sharing (n=18, 58.1%), whereas non-farmers more frequently preferred sharing with trusted partners (n=39, 63.9%) and were more likely to cite privacy and ownership concerns as the main barriers (n=31, 50.8%). Respondents perceived an overall positive ROI ($M=3.96 \pm 0.82$, $p < 0.001$; strong negative=1, neutral=3, strong positive=5) with no role differences ($p = 0.8$), although high cost remained the most frequently cited barrier to wider adoption. Overall, despite differences in monitoring priorities, governance transparency, and data-sharing perceptions, confidence in technological performance is shared. Keywords: Precision Livestock Farming, Multi-actor, Technology configuration, Data governance

Connecting the Dots: Interoperability from Regulation to Practice

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The final discussion of the "Connecting the Dots: Interoperability from Regulation to Practice" session will bring together perspectives from phenomics, digital infrastructures, and sector governance to address a central challenge: how to translate rapid advances in livestock data generation into practical, trusted and scalable use. Building on insights from contributions linked to HoliCow, EULIPHE and Digi4Live, this interactive conversation will explore how interoperability can be advanced across tools, platforms and stakeholders, and how regulatory and governance developments are shaping both the opportunities and constraints for data exchange. The panel will reflect on what is needed to connect data across systems and sectors while protecting legitimate interests, strengthening trust, and enabling fair and effective use of data for decision-making. This session fosters a focused exchange on how to move from fragmented data landscapes to workable pathways that support real-world implementation and sector-wide value creation.

Development of Sustainable Dairy Production in Germany - learnings from the "Dairy Sustainability Tool" project

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Since the 2010s, sustainability has gained increasing importance in the food value chain – in the production, processing and marketing of food, at national and international levels. This also applies to the German dairy industry. Were these sustainability initiatives focused mainly on the dairy processor level in the beginning, the focus progressively shifted along the whole value-added chain. Consumers, society, food retailers and food companies were wondering about the sustainability of milk production at farm level. Including German dairy farms in sustainability concepts poses particular challenges: the large number of farms, some with very different production conditions, the very broad range of sustainability aspects covering economic, ecological, social issues and animal welfare to be recorded at farm level and the fact that dairy farming has a strong linkage with the local environment. The problem: in the 2010s, there were scarcely any workable and cost-effective means to measure sustainability at farm level in its entirety. Against this background, a "basic-tool" for the sustainable development of dairy production in Germany was developed by actors from science and practice in an interdisciplinary approach. The aim was to develop a monitoring tool for dairy farms which measures and assesses a broad range of sustainability criteria in a large number of farms. It should provide information to business partners and society about dairy farms' strengths and weaknesses, and support evidence-based sustainable development of dairy farming. The sustainability criteria were screened for being a) scientifically defined and validated, and b) measurable and collectable at a reasonable cost and time by farmers themselves. To address the latter, mainly categorial indicators were used. The criteria were rated in four performance categories following their attribute value. These ratings based on scientific literature review, international frameworks, existing assessments of sustainability criteria in other measurement systems and relevant legislative frameworks. As a result, the tool consists of (a) a questionnaire covering more than 80 sustainability criteria in the dimensions economy, ecology, social aspects and animal welfare, (b) factsheets with explanations (scientific background and state of knowledge) for each criterion and its rating and (c) a central database. Since its introduction, over 15,000 dairy farms from about 30 dairies in Germany have participated. The project is fully funded by the participating dairies. Incentive schemes at farm level vary across dairies and range from no remuneration, through a flat-rate payment per completed questionnaire, to a condition for inclusion in bonus schemes. In all cases, remuneration is independent of the results; neither penalties nor performance-related fines are imposed. Farm benchmarking was provided to every participant to raise awareness and to serve as a continuous learning and development instrument. Regular reports allowed participating dairy processors to include the findings in the development of their own sustainability pathways and implement fact-based farmer communication and training. In-depth scientific analysis using multivariate statistical analyses informs both the public and the scientific community. After more than ten years, the project ends in 2026. We investigate the farm-level progress based on selected variables and identify persistent challenges in dairy production. Given the recent narrowing of the sector's sustainability focus to climate impact, we discuss lessons learned in the context of a climate-smart development of German dairy farms.

Outlook of a dairy farmer from Northern Germany: Strategies for resilient milk production

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Northern Germany is considered a favourable region for milk production in Northwest Europe. Comparatively, mild temperatures and favourable rainfall patterns ensure high and stable forage yields. However, milk production in this region also faces challenges: Intense competition for agricultural land has led to high lease and purchase prices for agricultural area. Farmers here underlay strict regulations with respect to nitrogen and phosphorus fertilisation. Also, in this region societal demands regarding animal welfare have increased, and a lack of sufficient dialogue has led to a decline in public acceptance of modern agriculture. Generally rising wages, exacerbated by a shortage of skilled workers, make it difficult to recruit and retain suitable employees. After a period of high milk prices, these have fallen again, and while production costs have remained high, many farmers are finding it difficult to operate profitably. Anticipating these long-term developments, the Grünhof farm's long-term strategy was to reduce feed and production costs and to become as independent as possible from the labour market. The central strategy is the combination of pasture-based milk production with high-performing animals, automatic milking systems, and a barn designed with animal welfare in mind. Grazing combined with high animal welfare standards ensures high public acceptance and also results in healthy, well-trained cows with few hoof problems, thus reducing veterinary costs. Consistent seasonal calving in autumn allows for targeted feeding of the animals for peak performance during the winter months, when the cows are kept indoors anyway. This also ensures healthy calves, as the calf barn is empty for periods of time, which reduces infection risks. The robust calves can be kept on pasture cost-effectively from their first year. The cows are turned out to pasture at the beginning of April. Day and night grazing is offered to the cows from early Mai to the end of September. Through targeted grazing management, pasture growth covers most of the demand for forage; only straw and limited amounts of concentrates are supplemented, thus reducing feeding costs in this period. The highly digestible, protein-rich pasture grass reduces the need for concentrated feed by 20% compared to the regional average. Twice a day, after visiting the milking robots, the cows receive access to a new area with fresh grass (AB system), thus motivating them to use the milking robot also during grazing season without spending extra time fetching them. Autumn calving allows for optimal utilisation of the generally higher milk prices in the winter months and gives the farm manager the opportunity to focus more on forage production and also to spend holidays and leisure time with the family during spring and summer, as there are no calvings or inseminations during this period, and no calves need to be fed. Instead of the otherwise typical regional strategy of increased herd sizes combined with high lease payments, Grünhof farm is generating a sufficient income with 140 high-yielding Holstein-Frisian cows based on only an equivalent of 2 full-time employees and comparably low feed costs. This approach allows for relative independence from the labour market and simultaneously frees up time for family and leisure. Grünhof acted as pilot-farm in the EU-Horizon2020-project R4D – Resilience for Dairy and a well reported example for future sustainable and resilient milk production.

Key developments and scientific focus fields in European dairy farming systems

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Food systems worldwide are undergoing major transformations driven by population growth, climate change, geopolitical tensions, and shifting societal expectations regarding sustainability, health, and ethics. The global demand for animal source food products is still rising. It is projected that global per capita consumption of animal-source foods will continue to increase, particularly in lower-middle-income countries. Also in Europe, livestock production is a major element of the food system. In 2024, approximately 41% of the estimated value of the European Union's agricultural output originated from animals and animal products. At the same time, livestock populations in the EU have declined by 8–16% over the past decade, reflecting structural adjustments driven by economic pressures, environmental regulations, and changing consumption patterns. In contrast, European poultry meat production is following an upward trend for the last 15 years. Dairy production in the European Union has expanded in the last decades, but in recent years stabilised at an annual level of about 160 million tonnes. It is expected that this consolidation will continue in the next few years and that transforming production systems towards increased sustainability and resilience will be a European focal point. In 2025, the European Commission (EC) presented its Vision for Agriculture and Food. The main objective for 2040 is an agri-food system that is attractive, competitive, sustainable and fair for current and future generations. To enable this, the EC puts research, innovation, knowledge and skills at the heart of Europe's agri-food economy. This presentation discusses current challenges related to the food system, the role of livestock within sustainable and circular agri-food systems, and the main trends shaping livestock farming in Europe from both practical and animal science perspectives. The future of European dairy farming systems will be the core element of the presentation. Key themes include climate-smart production, biodiversity, One Health, animal welfare, digitalisation, and incentives for change of human behaviour.

Which livestock systems best address both food insecurity and climate change? Comparing cattle, pig, poultry, rabbit, and snail systems in Cameroon.

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Ruminants play a central role in food security, rural livelihoods, and climate resilience across Sub-Saharan Africa (SSA), yet they also contribute substantially to agricultural greenhouse gas emissions and must adapt to increasing climate shocks and variability. While many studies assess productivity, resilience, and mitigation within ruminant systems, there is limited comparative evidence positioning ruminants within the wider livestock sector to clarify how their functions, benefits, and climate vulnerabilities differ from those of other species. The objective of this study is to apply the framework of Germer et al. (2023) to evaluate food security and climate-related outcomes of major livestock systems in Cameroon—specifically cattle, pigs, poultry, and selected non-conventional species (rabbits and snails)—and to clarify the distinctive role of ruminants within this broader context. Using farm-level survey data collected in 2022 in collaboration with Cameroon's Institute for Agricultural Research for Development (IRAD), we construct a structured set of locally validated, expert-informed indicators related to food security, climate vulnerability, and mitigation potential. The analysis spans multiple spatial scales, covering Cameroon's five agroecological zones, and major livestock production systems (pastoral, mixed crop–livestock, and more intensive systems). The selected indicators are designed to capture both short-term system responses to climate shocks and longer-term structural characteristics influencing adaptive capacity and sustainability trajectories. By situating ruminants within the broader livestock portfolio, this study provides comparative insights into how the ruminant sector contribute to food security, income stability, and climate resilience compares to other species, while also highlighting trade-offs in greenhouse gas mitigation potential. These findings support more informed livestock sector planning and identify pathways to strengthen the sustainability of broader food systems under a changing climate.

Current developments in the dairy sector in the USA

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The dairy sector in the USA continues to undergo tremendous change. This is evident by the rapid reduction in the number of dairy farms, and the growth of the average cow herd size. The average dairy farm now has approximately 400 dairy cows (not including youngstock), but it is quite common to see dairy farms with thousands or tens of thousands of dairy cows. Increasingly, dairy heifers are raised on specialized heifer raising ranches, often in the middle part of the country. Dairy herd sizes in the USA have increased greatly in the last 20 years. Milk production in this period has increased by 31%. While the national dairy herd has been varying between 9 000 and 9 500 thousand cows, the number of licensed dairy farms has decreased from 67 000 in 2004 to fewer than 24 000 in 2025. Consequently, the average herd size is now almost 400 dairy cows (excl. youngstock). There is barely a cap on the maximum herd size, and it is not uncommon to see herds with thousands of dairy cows. Dairy farms with more than 2 500 cows, which represent only 3% of all dairy farms, produce half of the milk in the USA. This consolidation is due to geography (wide open spaces and less strict environmental regulations in many states) and a culture where competition and expansion is welcomed and celebrated. One finds many abandoned older dairy farms. The reduction in the number of active dairy farms, and dairy farm families, means a strain on the viability of small towns in rural USA and a sense of loss of community. These farms are owned by fewer dairy farm families, who may own multiple farms in multiple states. These families may also have (co)ownership of processing facilities. Thus, the dairy sector follows the integration models of the poultry and pig sectors. The milk processing capacity in the country is growing strongly. Consolidation is also occurring in the allied dairy sector, where fewer but larger firms serve the dairy sector. Collectively, there remains a drive to become more productive, more efficient, more environmentally sustainable, and more profitable. Given the large variation in efficiency, some dairy farms are very profitable while many older, smaller, traditional operations struggle to remain viable. The lack of interest of the next generation to take over the dairy farm is often the main factor for farm closure. There remain strong research and investments in improved production, reproduction, genetics, nutrition, health, and labor management. Technologies driven by artificial intelligence are becoming more accepted. For example, these technologies aim to interpret the many data collected through wearable sensors, milking parlors, genomic testing etc. and offer actionable advice such as for animal treatment, replacement, and insemination decisions. Another example is the use of cameras to monitor worker task compliance, such as in milking parlors. The shortage of purebred beef owned by beef producers has led to a boom in the use of beef semen on dairy cows and a sharp increase in the use of dairy sexed semen. About 75% of all dairy heifer calves are born from sexed dairy semen. The price of 1-week old crossbred (beef-on-dairy) calves is more than US$ 2000 in some markets, prices that were unbelievable just a few years ago. The sale of these beef-on-dairy calves is now an important source of income for many dairy farms. Consequently, the number of dairy heifers produced has been reduced, which is leading to a shortage of heifers. It remains to be seen if this shortage will increase cow longevity. “Right sizing” animal inventories, which is the logistics of producing the right number of dairy animals at the right time, receives considerable attention. In conclusion, the US dairy sector is very dynamic with rapid changes, including adoption of modern technologies.

CO2 tax and N-legislation constrain Danish dairy production

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The Danish parliament decided in 2021 that Danish dairy farmers should decrease the enteric methane emission form their cows by 6% in 2025 and increasing to 25% in 2029. Furthermore, a newly established Green Deal in the Danish Parliament obliges Danish dairy farmers to pay a CO2-tax from 2030 on GHG-emissions from cows and their manure. New legislation from 2027 has the purpose to reduce N-leaching markedly by changing the N fertilization from an N-supply quota on crop level to an N-leaching quota on field level. This will especially impact Danish Dairy farms and have consequences for their roughage production, amount of purchased feeds and thereby ration composition. Eventually, it can also limit number of Danish dairy cows.

Regenerative agriculture as an evolving concept: emergent practices and farmer decision-making in Dutch dairy farming

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Regenerative agriculture (RA) is increasingly promoted as a response to pressing sustainability challenges, yet its loosely defined and context-dependent nature makes it both broad in appeal and difficult to study rigorously. To address this complexity, this research focuses on regenerative practices within Dutch dairy agriculture, a sector confronting urgent challenges related to biodiversity loss, soil quality, and nitrogen deposition. Specifically, this study aims to identify the emergent characteristics and applied practices of regenerative dairy farming, and to explore how farmer knowledge, discourse, and decision-making shape the transition towards RA. Data were collected through two rounds of in-depth interviews with 28 pioneering RA dairy farms participating in the ReGeNL transition programme, actively applying innovative or alternative practices that distinguish them from conventional Dutch dairy farming. The first round captured farm system characteristics, farmers' conceptualisation of RA, and the underlying reasons why farms are organised the way they are, including motivations and potential situational limitations. The second round gathered detailed dairy farm and herd management data, covering grazing strategies, feed composition, breed choices, and medical intervention plans. Notably, the pioneering farms interviewed tended to manage larger land areas with smaller herd sizes than the Dutch dairy average, reflecting a broader orientation towards extensification. Preliminary analysis of farm interviews revealed that farmers' understanding of RA centred primarily on strengthening natural closed cycles and reducing dependence on external inputs. Soil health was identified as the most important theme in transitioning towards RA, followed by animal health, welfare and production, with biodiversity and farm economics jointly ranked third. Beyond sustainability, farmers consistently cited ease of workload, economic considerations, and personal beliefs as defining factors shaping their management and farm design choices. The second round of interviews further revealed considerable variation in how these principles are translated into practice, spanning grazing strategies, breed composition, and feeding regimes, which reflects the context-dependent and emergent nature of RA. These findings highlight that the transition to regenerative dairy farming is deeply embedded in farmer identity, pragmatic constraints, and local context, consistent with the context-dependent nature emphasised in broader conceptualisations of RA. This conceptual complexity, and the considerable variation observed across farms, underscores the need for research approaches that link tangible farm-level outcomes with farmer discourse to offer a more complete understanding of RA's potential, limitations and trade-offs for the dairy sector.

Producer Mental Health and Wellbeing as a Critical Component of a Resilient and Sustainable Agricultural System: Insights from Canada

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The mental health and wellbeing of agricultural producers has emerged as a critical issue for the sustainability of the agricultural sector and all those who rely on it across the globe. Farming is widely recognized as a demanding profession characterized by economic uncertainty, long working hours, social isolation, and environmental and social pressures, all of which can contribute to stress, anxiety, and depression among producers. Studies in Canada and internationally suggest farmers face elevated mental health risks compared with the general population, highlighting the need for sector-specific understanding and support strategies. Canada's dairy sector presents a unique context in which to explore these challenges. Dairy farming systems in Canada vary widely in terms of scale, labour structure, technology adoption, and management practices. These differences can shape the daily experience of producers and influence both risk factors and protective factors associated with wellbeing. Understanding the factors that contribute to and alleviate stress, and how production systems interact with producer management practices and the health and wellbeing of producers is therefore essential to building resilience for livestock systems and the agricultural sector at large. This presentation will synthesize findings from Canadian research initiatives examining producer mental health and wellbeing and management practices, with a particular focus on dairy farmers. Drawing on national surveys of farmer mental health, sector-specific research, and studies examining structural and management practices and characteristics of dairy operations, the analysis highlights key stressors affecting producers and identifies factors associated with resilience and positive wellbeing outcomes. Evidence from Canadian studies indicates that producers experience high levels of stress related to the unpredictability of the agricultural sector, financial pressures, workload demands and the responsibility associated with managing family farms. At the same time, research suggests that farm structure and production systems can influence wellbeing in different ways. For example, labour availability, automation, herd size, and management intensity may affect workload, time flexibility, and social support networks. The presentation will also discuss how broader sector initiatives, including national research collaborations and producer wellbeing programs, are beginning to address mental health challenges in Canadian agriculture. By bringing together insights from several studies and initiatives, this work aims to highlight the importance of integrating human wellbeing into discussions of sustainable livestock production. Supporting the health and wellbeing of dairy producers is essential not only for individual and family wellbeing but also for the long-term resilience and productivity of dairy farming and the agricultural sector as a whole.This synthesis contributes to a growing body of knowledge on farmer wellbeing and provides insights relevant to researchers, policymakers, and industry organizations seeking to design effective, evidence-based supports for producers. Strengthening producer wellbeing must be recognized as a key component of resilient and sustainable agricultural systems.

Milk Quality Driven by Training Programs and Technical Support in Small and Medium Scale Dairy Farms

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The quality of milk is directly influenced by management practices, worker training, and the adoption of appropriate technical procedures. Continuous assistance and training programs have emerged as effective strategies to improve microbiological and compositional indicators and to support quality-based payment systems. This study aimed to assess, over a three-year period, the effects of training and monthly technical assistance on milk quality and on bonus indicators received by producers. The study was conducted on 44 dairy farms in Piracicaba, SP, Brazil, from 2017 to 2019. In the first year, farms were only monitored to establish a baseline for milk quality indicators. In the following years, a structured technical assistance program was implemented, including monthly visits, quarterly training sessions, and monthly reports with analytical results and management recommendations. Monthly bulk-tank samples were collected using bronopol vials for composition and somatic cell count analyses, and azidiol vials for standard plate count. All samples were processed following laboratory protocols, and quality-based payment was evaluated using the cooperative's bonus table. A mixed model with repeated measures was used, considering farm as a random effect and year as a fixed effect. The intervention significantly reduced standard plate count (-60%; $P<0.001$) and somatic cell count (-19%; $P=0.033$), while fat, protein, and SNF remained stable. Although overall bonus payments did not change, group-based analysis revealed important contrasts: producers who increased bonuses improved all components, raising the bonus from 2.3% to 5.2%, whereas those who reduced bonuses improved only SPC and SCC, with a decrease from 6.3% to 3.3%, indicating lower adherence to recommendations. Beyond farm-level improvements, the results highlight the broader impact of structured training and technical support on the regional dairy sector. By strengthening producers' technical capacity, promoting consistent adoption of best practices, and improving decision-making based on analytical feedback, these programs enhance production efficiency, reduce avoidable losses, and contribute to more stable and predictable economic outcomes. Such improvements are particularly relevant for small and medium-scale farms, which often face greater vulnerability to market fluctuations and sanitary challenges. From the consumer perspective, the widespread implementation of improved hygiene and management practices results in safer, higher-quality dairy products, reinforcing trust in the supply chain and supporting the long-term development of the sector. Moreover, as milk quality improves at the regional level, processors benefit from more uniform raw material, potentially reducing processing costs and increasing the value of final products. Overall, continuous technical assistance and periodic training led to meaningful improvements in milk quality, especially through reductions in microbial load and somatic cell count. When aligned with quality-based payment systems, these improvements contribute not only to the economic and productive sustainability of dairy operations but also to the broader resilience, competitiveness, and advancement of the regional dairy sector. (FAPESP PDIP 2017/50339-5)

Examination and comparison of measured and scored body conformation traits of German Holstein cows in 2015 and 2025

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Conformation traits in dairy cattle are usually recorded as a score from 1 to 9. These traits are typically assessed between the 50th and 100th day of lactation after the first calving. In 2015, in a project involving multiple breeding organizations, the conformation traits of approximately 1,200 Holstein cows were recorded after their first calving in Germany. In addition, eight traits were measured in 2015, six of which were also scored as conformation traits – such as body depth, rear udder height and rump width – and were therefore objectively recorded. In 2025, after approximately 10 years of genomic selection, during which genotyping of cows became standard practice, this objective recording of conformation traits was carried out again on approximately 2,100 Holstein cows, 1,871 of these could be used after a quality check. In 2025, 13 traits were measured, seven of which were also available as scored conformational traits, five of which were the same as in 2015. The differentiation ability (discriminatory power) of the trait scoring system was evaluated at the two time points separately (2015 and 2025) by comparing the LS-means of measured values across score levels, and together. LS-means for the scored traits rump width and body depth were significantly higher in 2025 compared to 2015, while those for rear udder height and median suspensory were significantly lower. The discriminatory power of the scoring system remained adequate, as LS-means mostly differed significantly between score levels for 2015 and 2025. Additionally, LS-means for both timepoints (2015 and 2025) were calculated to assess changes in the traits over the decade of selection. For most of the measured and scored traits, a significant increase in LS-means was observed from 2015 to 2025, reflecting consistent phenotypic changes over the ten-year period. However, these shifts do not necessarily imply optimality with regard to functionality or requirements of modern production systems. Finally, Pearson correlations between measured values and scores were calculated to determine the degree to which the scores reflected the actual trait variation. The correlations were all positive except for rear udder height and ranged between 0.41 and 0.59, or -0.56 for rear udder height in 2025, and thus remained almost unchanged compared to 2015. The observed phenotypic trends likely reflect broader breeding developments in dairy cattle breeding in Germany and other highly developed dairy regions, where genomic selection has accelerated genetic change and breeding goals increasingly emphasize functionality, udder health, and longevity alongside production traits. At this point and from a practical perspective, the results indicate that conventional conformation scoring systems remain a robust and informative tool for breeding programs and herd management even under ongoing genomic selection. At the same time, the moderate correlations between scores and objective measurements highlight the potential of more precise and automated phenotyping approaches to complement existing evaluation systems in the future. Overall, these results support the continued use of traditional scoring while encouraging the integration of high-resolution phenotyping technologies to further enhance dairy cattle evaluation and breeding strategies.

Evaluating greenhouse gas emissions of different breeding sheep pilot farm in Latvia

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Prudent use of natural resources is essential for the quality of life of inhabitants today, tomorrow, and for the future. The Intergovernmental Panel on Climate Change (IPCC) has developed guidelines for national greenhouse gas inventory and monitoring, which require extensive and reliable data. Previous studies on emissions produced by different species of agricultural animals, which form the basis of the IPCC guidelines, need to be updated to reflect the specific conditions of each country. Continuous and reliable data recording is required in all livestock sectors so these data can be used for various forecasts, including the calculation of greenhouse gas (GHG) and ammonia emissions under Latvian conditions. Data collection is an integral part of the development and updating of greenhouse gas inventories and must be adapted to Latvian conditions and periodically reviewed as part of the implementation of good practice. Currently, the volume of research on emission levels from individual agricultural animal species under Latvian conditions and within different livestock farming systems is insufficient. This research aims to verify the findings obtained from IPCC guidelines and related studies under practical farming conditions by monitoring the data required for inventory calculations on farms. Furthermore, the research aims to ensure the continued recording and accumulation of these data and knowledge so that the impact of greenhouse gas and ammonia emission mitigation measures can be incorporated into national emission inventory reports. In 2023, cattle are the largest source of methane emissions from enteric fermentation processes in Latvia, where sheep account for 2.0% of them. In the project, sheep farms in Latvia are divided into two categories according to the type of production: wool–meat farms and meat production farms. The first farm is actively involved in breeding activities, ensuring intensive indoor fattening of lambs of different sheep breeds under conventional or traditional (grazing) conditions. The lambs are fattened and sold for meat production. The fattening process is carried out under controlled conditions, recording all feed placed in the feeders and the amount consumed. The actual consumption of hay and straw cannot be precisely determined, as part of it is scattered on the pen floor and becomes bedding. The farm employs a breeding specialist and a veterinarian; lambs are weighed regularly, and their health is monitored. A study on feed utilization efficiency is also being conducted on the farm. The second sheep farm operates under an organic farming system and raises several sheep breeds. The farm is involved in a breeding organization; therefore, herd monitoring and control are carried out. The farm specializes in meat production. Pastures are intensively used for feed production, with efforts made to extend the grazing period as much as possible by growing catch crops and providing animals with cheaper available feed, mainly roughage from pastures. Regular monitoring of the herd's live weight is carried out on the farm. Sheep of all age and sex groups are weighed using electronic scales. Based on collected data evaluation of GHG from each farm was estimated by used in the country inventory methodology for different farming systems depending on local circumstances. Sheep breeding is not a big source of emissions in Latvia, but it is still important to evaluate its effect on the farm level.

Environmental Sustainability as a Dimension of Sheep and Goat Product Valorization: Preliminary Results from a Carbon Footprint Study of Greek Cheeses

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Sheep and goat dairy products play a crucial role in rural economies across Mediterranean regions, where traditional production systems and quality-label schemes such as Protected Designation of Origin (PDO) contribute to product differentiation and value creation. At the same time, increasing attention to environmental sustainability is reshaping the way these products are evaluated within agri-food systems and markets. Assessing the environmental performance of sheep and goat dairy products can therefore support both sustainable management practices and the valorization of traditional products. This study presents preliminary results on the carbon footprint of cheese production in a small-scale dairy plant in Greece, applying key methodological elements of Life Cycle Assessment (LCA). The case study concerns a dairy facility processing exclusively sheep and goat milk and producing several types of cheese, including PDO products such as feta and manouri, which represent important examples of differentiated small ruminant dairy products in Mediterranean markets. The assessment was conducted for a functional unit of 1 kg of packaged cheese, with system boundaries defined as "cradle to retail store" and a reference year of 2024. The analyzed stages include primary milk production, transport to the cheese plant, industrial processing, packaging, waste management, and distribution of the final product. The consumption phase and end-of-life disposal were excluded. Due to limited availability of primary data and the absence of access to commercial LCA databases, emissions were estimated using secondary emission factors derived from scientific literature and international guidelines. The results indicate a total carbon footprint of 15.42 kg CO_2-eq per kg of cheese. Primary milk production is the dominant stage, accounting for approximately 98% of total emissions (15.05 kg CO_2-eq/kg), confirming previous findings that farm-level processes largely determine the environmental profile of dairy products. In contrast, industrial processing and distribution contribute only about 2% of total emissions (0.37 kg CO_2-eq/kg). Within the dairy plant, energy consumption represents the main emission hotspot (78.40%), followed by packaging materials (19.71%), solid waste management (1.65%), and water use (0.24%). These preliminary results highlight the importance of farm-level management practices in shaping the environmental footprint of sheep and goat dairy value chains. At the same time, documenting the environmental performance of PDO cheeses can support their broader valorization by linking traditional quality schemes with measurable sustainability indicators. Despite several methodological limitations—such as the use of secondary emission factors and aggregated energy data—the study provides useful insights for identifying emission hotspots and guiding mitigation strategies. It also contributes to addressing a research gap regarding the environmental assessment of sheep- and goat-milk cheese production in small-scale Greek dairy plants.

Translational rumen microbiome research to improve cattle production sustainability

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Novel and adoptable technologies are needed to improveg feed efficiency while reduce enteric methane (CH4) emission in global ruminant production systems. Methane (CH4) is the second most important greenhouse gas (GHGs) that contributes to climate change, and it is the most significant source of emissions from ruminants in the Canadian agriculture sector. Ruminants have evolved to possess a diverse population of microbes in their rumen that can convert forage into nutrients for animal growth. This symbiotic microbiota can degrade most complex plant polysaccharides to produce short chain fatty acids, proteins, and vitamins, which satisfy the animal's energy requirement for maintenance and growth. However, this process also leads to the production of enteric CH4. There is growing evidence that composition and function of rumen microbiomes have both beneficial and detrimental impacts on cattle productivity, methane emissions, product quality and cattle health. This presents an opportunity to develop translational microbiome solutions to improve productivity and reduce the environmental footprint for cattle production. This presentation will introduce the recent research advances in rumen microbiome and discuss the opportunities to develop novel and industry adoptable microbiome solutions to improve feed efficiency and reduce methane emissions in beef and dairy cattle. Suchmanipulation solutions will lead to novel breeding and dietary supplementation strategies and targeted precision management to lower methane emission from dairy and beef cattle aligning with Canada's goal of Net Zero emissions from Agriculture by 2050.

Genome-wide analysis indicates host genetic control of ruminal microbial traits in Holstein cows

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The bovine rumen microbiota is a complex and dynamic ecosystem that influences many physiological processes, with major implications for cattle productivity and environmental sustainability. Emerging evidence indicates that host genetic variation influences the composition of ruminal microbes; however, the biological mechanisms and metabolic pathways that govern host regulation of these microbial communities are still not well understood. To address this deficiency, we evaluated host genetic control of ruminal microbial communities and their association with host performance by conducting genome-wide association studies (GWAS) on 593 microbial traits in 816 Holstein cows, using 4,549,172 imputed single nucleotide polymorphisms (SNPs) with imputation accuracy $r2 \geq 0.8$ and minor allele frequency ≥ 0.05. Microbial traits included clr-transformed abundances of six Rumino-signatures (RS), 118 microbial genera (102 bacterial, 10 archaeal, and 6 protist), and 469 amplicon sequence variants (439 bacterial, 8 archaeal, and 22 protist). We identified 5,201 significant SNP associations (q-value < 0.05), which clustered into 67 non-overlapping QTLs. Although associations were detected across all microbial domains, QTLs were domain-specific, with 54 linked to bacterial abundance, seven to archaea, and six to protists. Archaeal-associated QTLs predominantly involved members of the family Methanobacteriaceae, while Entodinium and Dasytricha were the most associated protist genera. Bacterial QTLs were mainly associated with the abundance of RS-RF16, Prevotella, Cryptobacteroides, Ruminococcus, Succinivibrio, Treponema, and members of the family Lachnospiraceae. Functional enrichment analysis of genes within QTLs identified pathways related to ion transport and neurophysiological signaling, including ligand-gated ion channels and calcium-dependent processes, as well as muscle contraction–related pathways. Gene ontology analysis further highlighted enrichment of synaptic signaling, regulation of cellular calcium homeostasis, membrane transport activities, and metabolic pathways involving NAD^+ and extracellular matrix components. Notably, pleiotropic associations of lead SNPs within QTLs were primarily observed with host performance traits including methane emissions, milk lactose content, and milk fat composition. In summary, our findings highlight the complexity and polygenic nature of the ruminal microbial ecosystem and suggest that shared host genetic factors may influence both microbial communities and host performance traits. Moreover, we identified candidate genes and pathways involved in host control of the ruminal ecosystem, with potential implications for hologenomics-based breeding strategies.

Host genomic influence on Gut microbiome diversity and composition in Atlantic salmon during Saltwater production phase.

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The gut microbiome influences nutrient utilization and resource efficiency in Atlantic salmon (Salmo salar). However, the extent to which gut microbiome diversity and composition are under host genomic control in salmon during the saltwater production stage is not well understood. The objectiveof this study was to estimate the additive genomic contribution to gut microbiome diversity and composition. Host genomic contributions to gut microbiome variation were evaluated in 425 Atlantic salmon. Distal intestinal digesta were collected postmortem when fish reached a mean body weight of 1,301.5 g. The fish population consisted of 30 full-sib families reared in eight tanks within a standard recirculating aquaculture system. Gut microbial communities were characterized by sequencing the 16S rRNA gene (V3-V4) region using Illumina MiSeq platform. Sequence data were processed using DADA2 pipeline, yielding 10,069 amplicon sequence variants (ASVs). Taxonomy was assigned using the SILVA v138.1 database (80% bootstrap). Fish were genotyped using AquaGen's custom Axiom® 56K SNP array, and 47,792 SNPs were used to construct the genomic relationship matrix. Genomic linear mixed models were fitted in ASReml 4.2 using REML to estimate additive genomic variance for each microbiome phenotype (alpha diversity, Faith's phylogenetic diversity, beta diversity, and individual taxon abundances). At the ASV level, heritability was estimated for most prevalent taxa (n = 243), and likelihood ratio tests were performed on each ASV additive genomic variance to identify heritable and non-heritable taxa. PERMANOVA was performed on unweighted and weighted UniFrac distances to test the effect of tank on microbial community structure. The gut microbiome of saltwater Atlantic salmon was dominated by Firmicutes (66%), followed by Proteobacteria (10%), Actinobacteriota (8%), and Bacteroidota (6%). At the genus level, Caldanaerobius (42%) was most abundant, followed by Romboutsia (8%), with other genera observed at lower relative abundances. Tank effects were significant but small, explaining 3.9% (unweighted UniFrac) and 3.4% (weighted UniFrac) of the variation. Heritability estimates for alpha (0.00-0.02) and beta (0.01-0.02) diversity phenotypes were low, with no significant genomic contribution. At the ASV level, heritability ranged from 0.00-0.12 (mean 0.02). Seven heritable taxa were identified: Caldanaerobius ($h^2 = 0.11$), Thermoanaerobacterium ($h^2 = 0.08$), Limnobacter ($h^2 = 0.07$), Candidatus Brocadia ($h^2 = 0.06$), Geminicoccaceae ($h^2 = 0.06$), Geitlerinema ($h^2 = 0.06$), and TM7a ($h^2 = 0.06$). Host genomic influence on gut microbiome diversity and community structure was low, with significant additive genetic variance observed only in a few taxa. These results indicate that microbiome phenotypes may exhibit lower heritability in aquaculture systems compared to what is observed in terrestrial livestock species. Keywords: Atlantic salmon, gut microbiome diversity, host genomic control, heritabilityof microbial taxa, microbiome phenotypes.

CowPi 2: Enhanced functional inference from rumen microbiome 16S rRNA data using a comprehensive genome database

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Background and objectivesMetataxonomic 16S rRNA gene sequencing remains the most accessible approach for characterising rumen microbiome composition, yet provides limited insight into functional capabilities. We previously developed CowPi, a rumen-focused implementation of the PICRUSt functional inference tool, which predicts microbial metabolic potential from 16S profiles using a reference database of ~500 cultured rumen genomes (Wilkinson et al., 2018, Front. Microbiol. 9:1095). Whilst CowPi demonstrated improved accuracy over generic PICRUSt for rumen samples, the limited genome database restricted taxonomic coverage and functional resolution. Here we present CowPi 2, which expands the reference database to 63,487 genomes and metagenomically assembled genomes (MAGs) from the HoloRuminant project, and implements the updated PICRUSt2 framework with improved phylogenetic methods and functional prediction algorithms. MethodsWe have constructed CowPi 2 using genomes and MAGs from the Hungate 1000 collection, BV-BRC, MGnify, and rumen metagenomic studies. The database encompasses eight ruminant host species sampled from ten body sites. All genomes underwent quality assessment using CheckM2, with stratification enabling selection of appropriate genome sets. Taxonomic classification employed GTDB-Tk, revealing representation across Bacteroidota, Firmicutes, Proteobacteria, Methanobacteriota, and Actinobacteriota. Functional annotation utilised eggNOG-mapper and Bakta. We extracted 16S rRNA genes using Barrnap, with recovery rates exceeding 90% in high-quality isolate collections and were used to generate a phylogenetic reference tree. Implementation follows the PICRUSt2 framework with improved hidden state prediction algorithms and expanded functional databases including enzyme commission numbers and MetaCyc pathways. ResultsCowPi-2 represents a 127-fold expansion in genome database size compared to the original implementation, with expanded taxonomic coverage including rumen-specific Prevotella lineages previously underrepresented in public databases. Quality metrics demonstrate variable completeness and contamination, with high-quality genomes (>90% completeness, <5% contamination) predominantly from isolate collections, medium-quality genomes (50-90% completeness, <10% contamination) from mixed sources, and draft-quality genomes from MAG collections. This stratification enables users to balance comprehensiveness against accuracy. Functional annotation shows expected enrichment in carbohydrate metabolism, energy production, amino acid metabolism, and cell wall biogenesis. The complete database, including quality assessments, taxonomic classifications, functional annotations, 16S rRNA sequences, metadata, and workflows, has been deposited in an open-access repository. Discussion and conclusionCowPi-2 leverages the improved hidden state prediction algorithms in PICRUSt2 with an extended ruminant-specific microbial genomic dataset. Validation studies with paired metagenomic and metatranscriptomic datasets have quantified improvements. This resource enables improved functional inference from ruminant-associated 16S rRNA datasets, facilitating investigation of rumen microbiome function in relation to animal productivity, health, and environmental sustainability, whilst providing a foundation for hypothesis generation to guide targeted metagenomic studies.

An Expanded Isolate and Genome Collection of Rumen Bacteria Reveals Microbial Signatures of Feed Efficiency in Cattle

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The rumen microbiome is a highly specialized anaerobic ecosystem that depolymerizes complex plant polysaccharides into fermentation products that fuel ruminant's metabolism and influence feed efficiency (FE). However, a mechanistic understanding of rumen microbial function has been limited by the scarcity of large, curated isolate genome resources. To address this gap, we present an expanded genomic resource comprising 1058 high-quality, non-redundant bacterial isolate genomes recovered from the rumen of dairy cattle. Based on Genome Taxonomy Database (GTDB) classification, this collection encompasses 59 bacterial families and includes 65 putatively novel species, substantially expanding the culturable diversity of the rumen-associated microbiota. Genome-resolved annotation revealed extensive diversity of carbohydrate-active enzymes (CAZymes) across taxa, with members of the Bacteroidota phylum encoding the highest proportion relative to total coding sequences of their genomes (~7%). Glycoside hydrolases (GH; 45%) and glycosyltransferases (GT; 33%) dominated the CAZyme repertoire, with GHs exhibiting the greatest family richness (>150 families). Notably, 39 of the predicted CAZyme proteins lacked detectable homology to CAZy or UniProt databases, suggesting putatively novel enzymes primarily identified within the genomes of Caldibacillaceae and Lachnospiraceae. Functional enrichment analysis indicated strong overrepresentation of pectin-targeting activities among CAZymes. To better resolve the genomic context of CAZymes and assess whether they are co-localized within metabolic gene clusters or encoded as discrete loci, we examined their distribution relative to reconstructed metabolic pathways. Across genomes, a large diversity of predicted metabolic pathways was identified, including 64 non-redundant characterized pathways associated with central energy-conserving processes, short-chain fatty acid production, amino acid metabolism, including thiamine-dependent pyruvate oxidation, succinate and propionate formation, and flavin-linked sugar catabolism. However, most CAZymes were not embedded within these pathways but instead dispersed throughout the genome, suggesting a modular organization of carbohydrate degradation and downstream metabolism. Further, to assess the potential of CAZymes as biomarkers of FE, metagenomic reads from an independent cohort of high- and low-FE cattle (n = 24 per group) were mapped to the non-redundant CAZyme catalog. No significant differences were observed in CAZyme abundance, richness, or diversity between groups, and machine learning models based on CAZyme profiles showed only a moderate discriminatory power for FE (AUC = 0.65). However, co-occurrence network analysis revealed distinct functional architectures. High-FE microbiomes exhibited tightly connected hubs centered on hemicellulose and pectin degradation (e.g., GH3, GH39, and Polysaccharide Lyase "PL" Family 12), whereas low-FE microbiomes showed greater centrality of enzymes associated with soluble and storage carbohydrate metabolism (e.g., GH35, GT1, and carbohydrate-binding module CBM48). Together, this work provides a high-resolution genomic framework for linking rumen microbial function to host performance, establishing a foundational resource for mechanistic studies and enabling microbiome-informed strategies to improve livestock productivity.

Persistent Auxiliary Microbiome of Early Colonizers Shapes the Developing Rumen Ecosystem

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The early life assembly of the rumen microbiome is a critical developmental process with lasting consequences for host physiology and greenhouse gas emissions. Using high-resolution longitudinal metagenomics in calves tracked from birth to three years (~800 days), we reconstructed 2,873 high-quality metagenome-assembled genomes, including 517 novel genomes predominantly detected in early life. These genomes, spanning 274 genera, define a diverse and functionally distinct auxiliary microbiome. Unlike transient early colonizers described in other ecosystems, this auxiliary community persists into adulthood, retaining ecological and functional relevance despite reduced abundance while contributing to the metabolic capacity of the mature rumen ecosystem. Temporal clustering revealed strong associations with dietary transitions and functional enrichments in environmental sensing, nutrient biosynthesis, and volatile fatty acid metabolism. Metabolic network analyses further demonstrated that auxiliary genomes complement non-auxiliary community members in key biosynthetic and fermentative pathways, indicating coordinated ecosystem-level interactions. These findings suggest that early colonizers act as ecosystem engineers, shaping the developmental trajectory of the rumen microbiome.

Effect of birth delivery method on microbial colonisation of key gastrointestinal regions in neonatal beef calves

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The microbial colonisation of the neonatal gastrointestinal tract is critical for digestive, immune and metabolic development. There is some evidence from human and rodent studies that Method of birth delivery can influence the initial process of microbial colonisation. The aim of this study was to investigate the effect of two birth delivery methods viz (i) transvaginal and (ii) elective caesarean section, on the microbial composition across key regions of the gastrointestinal tract of neonatal calves reared across two commercial research farms. Samples were collected immediately after birth from five gastrointestinal regions (Region); colon, ileum, jejunum, meconium, and rumen (swabs) from Aberdeen Angus calves delivered (Delivery) either transvaginally (TV; n = 11) or by caesarean section (CS; n = 11). Microbial DNA was extracted and subjected to 16S rRNA gene amplicon sequencing. Absolute microbial quantities were measured using 16S rRNA qPCR (Ct values). Alpha (Observed, Shannon, Simpson) and beta (PERMANOVA on Bray–Curtis dissimilarities) diversity were compared between delivery methods within each region, with sex and farm included as fixed effects. Differential abundance (Maaslin2) analyses were conducted with the same fixed effects. Alpha diversity varied significantly among gastrointestinal regions ($p < 0.05$) but did not differ between delivery methods within any region (all BH-adjusted $q \geq 0.78$). PERMANOVA indicated that gastrointestinal region strongly structured microbial community composition ($R^2 = 0.24$, $p = 0.0001$). A strong tendency towards a Region × Delivery interaction was observed ($R^2 = 0.057$, $p = 0.024$), indicating that any influence of delivery method was dependent on anatomical site rather than uniform across the gastrointestinal tract. Farm was not associated with statistically significant variation in community structure ($R^2 = 0.012$, $p = 0.17$), and no overall effect ($P > 0.05$) of delivery method across regions was detected. However, an analysis conducted per region detected no statistically significant treatment effects within individual gastrointestinal regions (all $p > 0.05$), suggesting that the interaction reflected modest, region-specific trends rather than strong site-specific effects. The early microbial establishment within the GIT of neonatal beef calves was therefore primarily driven by anatomical site rather than delivery method, with only weak, region-dependent differences associated with birth method. Microbial load and community composition were comparable between CS and TV calves at birth, indicating that initial gastrointestinal colonisation is largely independent of delivery method. These findings together with previous published data from our group suggest that postnatal environmental exposure and host–microbe interactions may play a greater role than birth method in shaping early gastrointestinal microbial assembly in beef calves.

Impact of pastures composition on calves microbiota in saliva, rumen and feces

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Rearing dairy male calves on underused pasture offers the opportunity to solve economic, sustainable and welfare associated tradeoffs. This study deals with assessing how different pasture compositions affect the calves microbiota across time. Understanding the influence on gastrointestinal settlement and development helps to understand ruminal fermentation efficiency. Three contrasting pasture compositions and one indoor system were compared by the effects on bacterial community composition. Buccal swab and feces sampling allowed repeated sampling over calves age (three- to six-month-old). Additionally, rumen fluid was collected once at the end of the experiment via esophageal probe. It is to be assumed that increase of composition and diversity of the pasture lead to higher microbial diversity. Male calves (n=72) were raised on three different composed pastures (intensive, permanent, alpine) and one hay based indoor system in Switzerland. Eighteen animals were examined per feeding system, every six were of the same genotype (Brown Swiss, Swiss Fleckvieh, Crossbreed = Limousin x Brown Swiss). Pasture composition was described by grass type and alongsides like herbs (%). Bacterial communities of buccal swabs (bioinformatically separated in oral- and rumen associated communities), rumen fluid, and feces were characterized by 16S rRNA amplicon sequencing, processed in QIIME 2 (DADA2), and taxonomically assigned using GTDB (v226). Diversity metrics (Shannon index and Bray–Curtis dissimilarities) and statistical tests (Kruskal–Wallis, PERMANOVA) were calculated in R (vegan package). Buccal swabs enabled us to collect rumen-associated samples at three time points on all locations. This challenging experimental design wouldn't allow an intensive sampling via the esophageal probe. In total 3,427 ASVs were revealed from buccal swab samples and 3,937 ASVs from rumen fluid at the end of the trial, with 1,726 ASVs overlapping in both sample sets and defined as rumen-associated ASVs in buccal swab samples. Thus, an impressive recovery of rumen bacteria could be shown using buccal swabs. As this method is less invasive and seems to be a robust method for future ruminant experiments. Microbial diversity analysis revealed the highest alpha diversity (Shannon) on almost all samples from animals at alpine pastures, followed by the permanent pasture. Beta diversity analysis showed a clustering of the indoor samples with intensive, and permanent with alpine. Changes according to the age of animals were minor while showing only no or minor significance for the diversity structure. Calves grazing alpine and permanent pastures developed richer, more diverse microbiota compared to indoor or intensive systems. In conclusion, our results support the hypothesis that greater diversity in pasture composition is associated with higher diversity and complexity of microbial communities in calves. In addition, we assume that buccal swab sampling provides a good approximation on rumen fluid microbial community.

Multi-block sPLS-DA Shows That Plasma Metabolomics Most Accurately Classifies Piglets Across Production Chains than fecal metabolomics and metagenomics

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Omics technologies offer powerful approaches to characterise complex biological systems and to uncover biomarkers associated with health, disease, and performance. They also hold considerable promise for describing animal production environments, where pigs are exposed to interacting management- and environment-related factors, including housing conditions, sanitary status, and antimicrobial usage, all of which may influence disease susceptibility. Given the substantial costs associated with omics analyses, determining which omics layers most effectively capture biologically relevant variation driven by management practices is of practical importance. In this study, we applied an integrative multi-omics strategy to investigate biological responses to contrasting management systems in pigs and to compare the discriminative ability of different omics datasets. Forty piglets originating from the same farrowing unit were assigned to two swine production chains (SPC1 and SPC2) that differed in housing conditions and antimicrobial protocols. Animals were monitored from weaning to slaughter. Blood haematology, plasma metabolomics, faecal metabolomics, and faecal shotgun metagenomics were collected two weeks post-weaning (T1), seven weeks post-weaning (T2), two weeks after transfer to growing–finishing facilities (T3), and at slaughter (T4). Growth performance did not differ between production chains at any sampling point ($P > 0.05$), whereas diarrhoea incidence was significantly higher in SPC2 at T1 ($P < 0.05$). Multi-omics datasets were integrated at each time point using DIABLO (Data Integration Analysis for Biomarker discovery using Latent Components). Across time points, faecal microbial functional profiles (KEGG orthologs) accounted for the largest share of variance in the discriminant components (up to 0.45 at T2), suggesting a sustained influence of management conditions on microbial metabolic potential. In contrast, plasma metabolomics consistently achieved the lowest classification error rates and highest AUROC values, outperforming both microbial taxonomic and functional profiles. At the early post-weaning stage, group separation was primarily driven by inflammatory and immune-related haematological markers, along with microbial metabolites derived from amino acid metabolism, particularly in SPC2. At later stages, discrimination was mainly associated with plasma metabolites related to lipid metabolism, xenobiotic processing, and energy pathways, together with faecal metabolites involved in carbohydrate and aromatic compound metabolism. In conclusion, this study demonstrates that plasma metabolomics is the most informative omics layer for discriminating farming conditions and capturing management-driven effects in pigs. Among the evaluated omics layers, the systemic metabolome consistently showed the strongest discriminative performance across time points, indicating a high sensitivity to differences in management practices under field conditions. This was particularly evident during the post-weaning and growing phases, when physiological responses to environmental and nutritional factors are most pronounced. These findings highlight plasma metabolomics as a robust systemic indicator of management effects, reflecting the downstream physiological consequences of farming conditions by directly capturing host metabolic responses it provides a reliable framework for identifying biomarkers of management practices and for linking interventions to animal physiology

Genome-Resolved Profiling of an Expanded Swine Gut Isolate Collection Reveals Functional Signatures of Health and Disease

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High-resolution genome data are critical for resolving the functional roles of swine gut microbes. However, many swine-associated lineages remain uncultured or underrepresented in reference databases, limiting our ability to link microbial functions to host traits. Although culture-independent metagenomics provides valuable community-level insights, it often lacks the taxonomic resolution required to assign functional potential to specific organisms, thereby constraining the translation of microbiome associations into actionable strategies. This limitation is particularly consequential for post-weaning diarrhea (PWD), a major challenge in swine production that results in substantial economic losses and increased antimicrobial use. Understanding the functional capacity of gut microbes and identifying health-associated microbial features is therefore critical for the development of microbiome-based interventions to promote gut health and reduce disease susceptibility. Here, we constructed an integrated genomic resource of the swine gut and fecal microbiota comprising 300 high-quality, non-redundant bacterial isolate genomes recovered from pig gastrointestinal tract. The isolate collection encompasses 33 bacterial families, featuring several previously uncultured representatives of the Lachnospiraceae, Lactobacillaceae, Desulfovibrionaceae, Clostridiaceae, Streptococcaceae and other phylogenetically diverse lineages. Notably, the collection includes 55 putatively novel species not represented in current reference databases. Functional profiling of the isolate genomes revealed extensive diversity among both known and previously uncharacterized metabolic gene clusters, biosynthetic gene clusters (BGCs), and antimicrobial peptides (AMPs). To assess the ecological and clinical relevance of these features, we analyzed their distribution across the microbiota of pigs with and without post-weaning diarrhea. Metagenomic read recruitment from independent swine cohorts against the isolate genome database showed that key functional features, particularly BGCs and AMPs, robustly discriminated between healthy and diarrheic pigs, with significantly reduced abundance in PWD-associated host microbiomes. Several taxa, including Aneurinibacillus, Blautia, Dorea, Prevotella, Paratractidigestivibacter, and Parabacteroides emerged as dominant contributors to health-associated functional traits. Combining high-throughput culturomics with machine learning-based functional inference, this study establishes an expanded swine gut isolate genome resource and identifies microbial functional signatures linked to host health. Our findings highlight the biosynthetic potential of secondary metabolites and antimicrobial peptides as key determinants of gut resilience and provide candidate microbial biomarkers and therapeutic targets for improving resilience to post-weaning diarrhea in sustainable swine production systems.

Characterization of the core porcine microbiota across production phases, animal tissues and genetic lines in an integrated production system

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The microbiome constitutes a complex and dynamic ecosystem that plays a central role in digestive efficiency, immune modulation and reproductive performance in pigs' production. While most studies focus on the gut, a holistic, longitudinal characterization across multiple body sites and production phases within a single integrated system is essential to identify critical windows of instability and opportunities for intervention. The aim of this study was to characterize the structure, diversity and stability of porcine microbiota from sows to finishing pigs within an integrated production system. We evaluated the effects of productive phase (gestation, farrowing, weaning, fattening), sample tissue (faeces, vagina, mouth, skin and nasal mucosa), farm of origin and genetics (purebred vs. hybrid) .We hypothesized that (i) tissue type and transition periods are the main determinants of microbial structure, and (ii) farrowing and weaning/feed transitions represent phases of reduced stability and increased variability. High-throughput 16S rRNA gene sequencing was performed in three complementary cohorts:(1) sows (n=453; fecal and vaginal samples collected during insemination, gestation and peri-partum; 47.1 million sequences); (2) piglets (n=270; mouth, feces and skin samples collected from 72 h post-birth through weaning and starter phases; 27.9 million sequences); and (3) wean-to-finish pigs (n=99; mouth, feces and skin; 7.8 million sequences). Alpha diversity indices, beta diversity (PCA, PLS-DA, permanova), taxonomic profiling at phylum and genus level, and determination of the core microbiota were performed using R (v3.6), Excel and JMP programs. Core populations were defined as species present in ≥85% of samples (sows) or ≥90% samples (wean-to-finish). In sows, vaginal microbiota displayed lower alpha diversity and a markedly smaller core (25 species representing 41.7% of sequences) compared to faeces (138 species; 74.5%). The post-partum period was characterized by significantly reduced diversity ($P<0.001$), higher dispersion in beta diversity analyses and pronounced alterations in the Bacillota:Bacteroidota ratio, indicating microbial instability at farrowing. Farm of origin influenced vaginal communities more strongly than fecal ones. In piglets, sample type was the main source of variance. Skin exhibited the highest richness (277 core species) and greater evenness compared with mouth (63) and feces (49). Diversity increased progressively with age, reflecting microbial maturation, but a transient decrease was observed at weaning, particularly in gut and oral samples. Farm of origin significantly shaped community structure (PERMANOVA $P<0.001$), especially in skin. ##In wean-to-finish pigs, feed transitions induced short-term fluctuations in alpha diversity, mainly in feces and skin, followed by stabilization. Genetic background exerted moderate effects: hybrid pigs showed greater variability in fecal and skin microbiota compared to purebreds. Across tissues, core microbiota accounted for 80–92% of total sequences, indicating a relatively stable microbial backbone. As conclusion, farrowing and weaning/feed transitions represent critical windows of microbial instability and increased dispersion. Farm environment and diet are dominant drivers, whereas genetics exerts a moderate, tissue-dependent influence. Systematic monitoring of these transition periods may provide a basis for targeted nutritional, management and sanitary strategies aimed at enhancing health, resilience and production performance.

Variability of vaccine responses in laying hens: assessing the holobiont

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Vaccination is essential for poultry health, yet marked inter-individual variability in vaccine responses challenges disease control and productivity. Both host genetics and gut microbiota are known to modulate immunity, but their relative contributions to vaccine responsiveness in laying hens remain unclear. This study aimed to quantify genetic and microbial influences on humoral and cellular immune responses to vaccines. A total of 552 White Leghorn hens from a single line were raised under controlled conditions to 22 weeks. Birds were genotyped using a 55K SNP array to estimate heritability and perform genome-wide association studies (GWAS). Humoral and cellular immune responses were evaluated following vaccination against newcastle disease virus (NDV), infectious bronchitis virus (IBV), avian encephalomyelitis virus (AEV), and avian pneumovirus (APV), using antibody titers and enzyme-linked immunospot assays. Caecal microbiota was characterized at 22 weeks using 16S rRNA sequencing, and analyses were performed on rarefied amplicon sequence variant (ASV) counts. Marked variability in vaccine responses was observed, with a mean proportion of birds failing to mount detectable antibody responses between 22.7% and 76.5%. Heritability estimates among responders for antibody levels treated as a continuous trait indicated null-moderate genetic influence (0.00–0.39), whereas analysing vaccine response as a binary trait distinguishing responder from non-responders revealed a moderate genetic contribution (0.22–0.40). Genetic and phenotypic correlations showed complex relationships among vaccine traits, including weak positive and negative correlations across vaccines and time points. GWAS identified significant QTLs on chromosomes 1, 4, 5, 8, 11, 15, and 28, with functional annotation highlighting genes such as MKNK1 and TRABD2B, and the non-coding RNA vaultRNA potentially contributing to immune variation. Microbiability estimates for vaccine response traits ranged from 0.10 to 0.25, indicating a contribution of caecal microbial composition for both continuous traits among responders and traits distinguishing responders from non-responders. Preliminary microbiome-wide associations studies (MWAS) revealed ASVs associated with the responder versus non-responder trait across NDV timepoints, while associations for the continuous trait appeared only for cellular immune response among responders. Overall, these findings demonstrate that host genetics and gut microbiota jointly shape vaccine responses in poultry. Moderate heritability supports the feasibility of genetic selection, while microbiability highlights substantial microbial contributions. Integrating genomic and microbiome data provides a framework to understand immune variation and to design breeding strategies combining genetics and microbiota modulation. Ongoing holobiability analyses will further quantify the combined and interactive effects of the host–microbiota system, advancing holistic approaches to improve poultry vaccine responsiveness and resilience.

Microbiota dynamics and core taxa in milk, teat skin, and feces of cows with and without mastitis

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Mastitis is one of the most common and economically significant diseases in dairy production. Yet the temporal dynamics of milk, teat skin, and fecal microbiota surrounding mastitis onset remain incompletely understood. We performed a longitudinal study of 21 Nordic Red primiparous cows by weekly sampling of milk, teat skin swabs, and feces during the lactation. Twelve cows remained mastitis-free, whereas nine experienced at least one case of mastitis. Based on the health records, 373 milk and teat swabs, as well as 295 fecal samples were selected for 16S rRNA gene amplicon sequencing (primers 515F/806R). Reads were denoised to amplicon sequence variants (ASV) in Qiime 2 and taxonomically classified with SILVA 138.1. Milk bacterial community of healthy cows was highly diverse, comprising 612 genera across 28 phyla, with Firmicutes, Actinobacteriota, Proteobacteria, and Bacteroidota being most abundant. While relative abundance fluctuated across lactation, alpha diversity remained largely stable. Indicator species analysis revealed 32 ASVs associated with mastitis-free cows and 65 with mastitis-affected cows; healthy cows showed higher relative abundance of Lactiplantibacillus spp., whereas mastitis-associated samples exhibited elevated levels of Staphylococcus, Streptococcus, and Escherichia-Shigella. Bray–Curtis dissimilarities revealed no significant differences in overall community structure between affected and healthy quarters from the same cow (adonis P=0.32), suggesting that udder-level microbiota shifts precede quarter-specific clinical symptoms. Teat skin microbiota was composed of 29 phyla and was dominated by Firmicutes, Actinobacteriota and Bacteroidota, with stable core genera (Lachnospiraceae NK3A20 group, Oscillospiraceae UCG-005, Corynebacterium, Romboutsia and Methanobrevibacter) observed across lactation. Although indicator ASVs distinguished mastitis-free and mastitis-affected cows, overall taxonomic composition remained similar between groups. Fecal microbiota showed high stability across lactation and was dominated by Firmicutes and Bacteroidota. Core microbiome analysis demonstrated shared ASVs between milk, teat skin, and feces, indicating potential microbial exchange between host body sites and environmental reservoirs. The findings underscore that mastitis-associated dysbiosis is detectable in milk shortly before clinical diagnosis, that microbiota shifts occur across the entire udder rather than being confined to the infected quarter, and that overlapping core microbial populations across sample types reflect interconnected microbial ecosystems. Together, these results advance understanding of host-associated microbiome dynamics regarding mastitis occurrence.

Rumen colonization and succession of microbial guilds during lamb development is affected by the presence of the dam

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The health and development of ruminants are largely influenced by their microbiota, particularly the rumen microbial community, which is shaped by early-life factors and microbial colonization processes. In many sheep production systems, lambs are separated from the dam shortly after birth and reared with artificial milk to optimize labor and milk production. However, this practice may negatively affect lamb growth and health. In this work, we investigated the effect of maternal presence on the rumen microbial composition and its temporal dynamics in lambs from birth to the end of the growing period. We also assessed a method of identifying microbial groups of ecological and phenotypic significance while reducing dataset complexity, allowing improved classification of the microbiota and tracking microbial changes during early life. The study involved 19 maternal-reared lambs and 13 artificially-reared lambs without adult contact, all weaned at 7 weeks of age. Rumen contents were sampled weekly from birth to 10 weeks and once more at slaughter (17 weeks). Non-negative matrix factorisation was used to analyse the relative abundance of ASVs revealed by metataxonomic analysis, in order to identify bacterial guilds called RuminoSignatures (RS). We found that the rumen bacterial community was structured into distinct RS whose temporal dynamics varied according to rearing system and feeding conditions. Some RS predominated shortly after birth, while others increased later during the suckling period, revealing a clear temporal succession in the establishment of rumen microbial taxonomic guilds during early life. Overall, RS trajectories were influenced by both age and rearing method, suggesting that maternal presence may shape specific patterns in bacterial guild prevalence. As a perspective, we are currently evaluating the prediction of microbial guilds in ewes before lambing and at weaning to gain further insight into potential maternal influences on rumen microbial establishment in lambs. In addition, metagenomic analyses are being conducted to infer functional capacities and link microbial functions to the taxonomic profiles identified.

Associations between rumen and faecal microbiota and inflammatory status in transition dairy cows

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The transition period around calving is critical for dairy cows, due to the significant physiological, metabolic, and nutritional challenges. Growing evidence suggests that inflammation can be a major factor in transition cow health. Although a physiological inflammatory response occurs in all cows after parturition, it generally diminishes within the first three weeks postpartum. However, some animals undergo prolonged inflammation, which could negatively impact cows' health, fertility and milk production. Although the role of gut microbes in inflammation is well established in human studies, it has not been sufficiently researched in ruminants, particularly during the transition period. To investigate the relationship between inflammatory status and gastrointestinal microbial composition, 60 multiparous dairy cows from two research farms were monitored during the transition period. Blood samples collected at 21 days postpartum were analyzed for inflammatory biomarkers (serum amyloid A [SAA], haptoglobin [Hp], and albumin: globulin ratio [A: G]) and metabolic biomarkers (insulin, glucose, beta-hydroxybutyrate [BHBA] and non-esterified fatty acids [NEFA]). Rumen and faecal samples collected at 21 days postpartum and faecal samples collected 7 days prepartum were subjected to metataxonomic analysis targeting bacteria, archaea, protozoa, and fungi. Based on inflammatory biomarkers, cows were classified into two clusters: acute phase response plus diseased cows (APR+, n=20) and non- acute phase response (Non-APR, n=34) cows. Dry matter intake was lower in APR+ cows, whereas milk yield did not differ between clusters. All inflammatory biomarkers differed between clusters, while among metabolic indicators, only NEFA concentrations were increased in APR+ cows. Microbial analyses showed that rumen bacterial and protozoal communities differed between inflammation clusters, with archaeal composition showing a tendency toward a difference. In contrast, faecal microbial communities were not affected by inflammatory status either pre- or postpartum. Distance-based redundancy analysis (db-RDA) indicated that the farm was the primary driver of variation in both rumen and faecal microbiota. After accounting for farm effects, blood inflammatory and metabolic variables explained a modest but sometimes significant proportion of microbial variation. The A: G ratio was associated with differences in rumen bacterial and archaeal communities, while SAA was associated with variations in rumen fungal and protozoal composition. Differential abundance analysis revealed several rumen and faecal bacterial and archaeal taxa that varied at the amplicon sequence variant (ASV) and genus levels between clusters, indicating their potential as indicator species of inflammatory status. Accordingly, inflammation at 3 weeks after parturition is associated with shifts in the rumen but not the faecal microbiome. Although farm effects remain the dominant source of variation, the rumen microbial structure show links with some blood inflammatory biomarkers. This highlights the potential interplay between host inflammatory status and rumen microbial composition in transition dairy cows.

The effect of fat type and inclusion level on the rumen microbiome and methane in dairy cows

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Dietary fat inclusion is a way to reduce enteric methane (CH4) emission in dairy cows. Different fat types contain different proportions of fatty acids (FA), however, the effects of fat types and their levels of inclusion on the rumen microbiome are poorly understood. The aim of this study was to test the effect on rumen microbiota (Bacteria, Archaea, Fungi and Protozoa) when cows were fed either a diet including rapeseed (RS) at 3 different inclusion levels (low=LR, mid=MR and high=HR) or palm kernel oil (PK) at 2 different inclusion levels (low=LP and mid=MP) or no fat (control=CO). The trial was run with 24 multiparous Holstein cows, 4 per treatment in each of 6 periods of 21 days each, with 6 treatments in a 6×6 Latin square design (N=144). Gas production and milk composition was determined. Amplicon sequencing was conducted on rumen samples for four microbial target groups, namely; Bacteria, Archaea, Protozoa and Fungi using specific primer sets. Analysis for each microbial target group included microbial composition, diversity and differential abundance. Dietary supplementation with RS at mid or high dose or PK at both doses was effective at reducing CH4 intensity (CH4/ECM) and yield (CH4/DMI). Low to mid levels of RS had a positive effect on ECM whereas PK had negative effect, particularly in the case of MP. Palm kernel oil had the greatest reduction in CH4 production at the mid dose. However, after accounting for the associated decrease in ECM, the reduction in CH4 intensity (CH4/ECM) was slightly lower than that observed for the high RS treatment (HR, 14% vs MP, 13% reduction). Rumen communities were dominated by (genera >10% reads) Prevotella, UBA2810 (Bacteria), Methanobrevibacter and Methanosphaera (Archaea), Neocallimastix, Piromyces, NY05 and NY09 (Fungi) and Entodinium, Epidinium and Isotricha (Protozoa). RS showed minor effects on the rumen microbial diversity while larger shifts were seen with PK especially in cows fed MP. Rumen Bacteria and Fungi compositions were most affected by PK, whereas the archaeal community showed comparatively smaller changes. Bacterial and archaeal richness was reduced in MP compared to all other diets, fungal richness was reduced in LP compared to all other diets and the protozoal richness was not affected by diet. Compared to the control RUG420 (Acutalibacteraceae) increased with both RS and PK diets but was the only bacterial genus detected differentially abundant across all RS levels, while 14 and 41 additional genera differed in the low and mid PK diets, respectively. For archaeal taxa, 1, 4, 1, and 6 genera were found differentially abundant in MR, HR, LP and MP diets, respectively. Methanarcanum decreased with MR, HR and MP diets, Methanosphaera increased with HR, LP and MP, and Methanobrevibacter increased in HR and MP, compared to CO. The fungal genus Buwchfawromyces dramatically increased (16-27 fold change) with all but the LR diet. Additionally, PK diets had an increase in Neocallimastix and a decrease in NY05 and NY09. RS diets had 1 differentially abundant protozoa genus at the mid (Eudiplodinium) and high (Entodinium) levels, while the PK diets resulted in a decrease in Dasytricha plus 3 additional changes at the mid levels. In conclusion, PK resulted in a greater disruption of the microbiome and the largest drop in CH4 production but was accompanied by a drop in DMI and ECM. Low RS had minimal effects on the rumen microbiome, DMI and ECM but also CH4. Mid and high levels of RS resulted in less changes to the rumen microbiome than PK and reduced CH4 measures linearly, however, feeding high RS did not provide additional ECM yield.

Effects of Heat Stress on gut microbiota in Iberian and crossbred pigs

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Heat stress (HS) in pigs reduces feed intake, productive performance, and protein and energy metabolism, while increasing water intake to maintain body temperature within physiological ranges. These physiological changes may alter the composition and functionality of the gut microbiota. In addition, elevated temperatures can disrupt gastrointestinal homeostasis, potentially leading to dysbiosis, which is associated with impaired nutrient absorption and immune suppression. The aim of this study was to evaluate the effects of prolonged HS exposure on hindgut microbial composition and predicted functionality in growing pigs, and to assess potential differences between two distinct genotypes. A 2×2 factorial experimental design was used, including 11 commercial crossbred pigs (CO; Pietrain × (Landrace × Large White)) and 11 Iberian pigs. Animals were exposed to either thermoneutral conditions (TN; 23.5°C) or heat stress (HS; 33.5°C) for 28 consecutive days. At the end of the experimental period, pigs were sacrificed and colonic contents were collected, snap-frozen in liquid nitrogen, and stored for further analyses. Microbial community composition was assessed by amplification and sequencing of the V3–V4 regions of the 16S rRNA gene using the Illumina MiSeq platform. Taxonomic assignment was performed using a Bayesian classifier (Wang et al., 2007) trained with the SILVA database version 138 (99% OTUs full-length sequences) (Pruesse et al., 2007). Alpha diversity indices (Shannon, Simpson, richness, and evenness), beta diversity analyses (PCA, PLS-DA, and permutational analyses), and taxonomic profiling at phylum and genus levels were conducted using R (v3.6), Excel, and JMP. Functional potential was predicted using PICRUSt2 (Douglas et al., 2020). No significant differences were observed in alpha diversity between the main factors, although a significant interaction was detected for Pielou's evenness index ($P < 0.05$). Permutational analysis revealed differences between breeds ($P = 0.042$), but not between thermal treatments, suggesting a possible long-term adaptation of hindgut microbiota to HS in both genotypes. Likewise, no differences were observed in the relative abundance of the main bacterial phyla. Functional prediction analysis identified differences between breeds in 21 metabolic pathways, mainly related to energy metabolism (NADPH2 dehydrogenase, sucD, HypD), carbohydrate metabolism (pfkC, dhaB, rbsD, epsF), cell envelope processing (LptD, LptA), and bacterial immunity (csm1, csm2, csm4, csm5). Heat stress only affected the NADPH2 dehydrogenase pathway. As conclusion, prolonged exposure to heat stress appears to have a limited effect on hindgut microbial composition and predicted functionality, possibly due to the ability of microbiota to adapt and recover under these conditions. However, differences between genotypes suggest that host genetics may influence gut microbial composition and metabolic potential, reflecting underlying physiological, metabolic, or behavioral differences. The PIGWEB project has received funding from the European Union's Horizon 2020 research and innovation programme (grant agreement No. 101004770).

Utilization of free amino acids in broiler chickens revisited

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The inclusion of free amino acids (AA) in feed formulation enables precise adjustment of individual dietary AA concentrations to meet animal requirements while simultaneously lowering the crude protein (CP) content. This approach reduces nitrogen (N) intake and excretion without compromising performance. Hence, the environmental impact of animal production is mitigated and the efficiency of animal-derived food production is enhanced by increasing nitrogen utilization efficiency (NUE). The need to include free AA rises as the dietary CP level decreases. Absorption processes in the intestine differ between peptide-bound and free AA. Several studies in various species, including poultry, have shown that free AA are absorbed more rapidly into the systemic circulation than peptide-bound AA. With different absorption rates, AA may be available in an unbalanced pattern for protein synthesis even though dietary AA supply is formulated as balanced. The dietary inclusion of free AA apart from which utilization is impaired seems to be higher than previously assumed. In a recent research project, incremental substitution of peptide-bound with free AA was investigated in very low-CP diets (~16.5% for birds up to 3 weeks of age) that remained adequate in all AA concentrations. This required the use of 19 g free AA/kg of diet. Soy protein isolate served as the source of peptide-bound AA, as it provides protein with minimal amounts of other nutrients. The digestible AA from the soy protein isolate were substituted with all 20 proteinogenic AA in free form, keeping digestible AA concentrations constant. Performance remained unaffected at inclusion levels up to 54 g free AA/kg of diet—considerably higher than current standards in commercial feed formulations. Results of experiments on the utilization of free AA in diets may be confounded by affected feed intake after a change to diets high in free AA. In a complementary experiment, the immediate effects of changing to diets high in free AA were investigated over time using an almost identical feed composition. Feed intake was affected for 3 days after diet change. This impacted on the determined weight gain, gain:feed, and NUE—likely reflecting delayed responses to the previous day's intake. This temporary reduction in feed intake may have been associated with a shift in acid-base balance toward acidity. Reasons for this shift include an acidifying effect of AA degradation and the hydrochloride of l-lysine·HCl, which increased the acid load due to HCl concentrations with rising substitution of peptide-bound by free AA. Whether the altered acid-base balance was a cause, consequence, or unrelated correlate of reduced feed intake remains unknown. Adaptation to high dietary inclusion levels of free AA appeared to occur mainly within the first 3 days after diet change. Indicators of acid-base status in blood, plasma AA concentrations, and the number of altered metabolites detected in untargeted metabolomics analyses all declined or normalized thereafter, while NUE remained unchanged. The unaffected NUE after 3 days of adaptation suggested an equal efficiency of accretion of free or peptide-bound AA. Hence, plasma AA concentrations seem unsuitable as indicators for AA utilization because NUE, being the most reliable key figure, was unaffected. In summary, results of a series of experiments gave no evidence for different utilization of peptide-bound and free AA despite of different absorption processes. While an upper threshold for dietary free AA inclusion was found, it is considerably higher than current commercial practice. Reduced feed intake seemed to be the main cause for reduced performance above this level.

Effects of controlled lactic acid bacteria fermentation on physicochemical properties of rye and wheat grains

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Cereal grains such as rye (Secale cereale) and wheat (Triticum aestivum) are widely used in animal nutrition. However, the nutritional utilization of certain feedstuffs in poultry may be limited by anti-nutritional factors such as non-starch polysaccharides (NSP) resulting in high digesta viscosity, which can negatively affect nutrient digestibility. Fermentation with lactic acid bacteria (LAB) has been proposed as a promising strategy to modify physicochemical properties of cereal grains and improve their suitability as feed ingredients.The aim of the present study was to evaluate the effects of LAB fermentation on three rye cultivars (A, B, C) and wheat as control. Fermentation was carried out using three LAB strains: Lactiplantibacillus plantarum, Limosilactobacillus fermentum and Pediococcus acidilactici. Dry matter content was set to 62.5%. Eight experimental treatments were applied for each cereal: individual inoculation of each LAB strain, three combinations of two strains, a combination of all three strains, and a non-inoculated control. Every treatment was prepared in three independent replicates twice (a1+2, b1+2 and c1+2). Total LAB counts (CFU/g), pH values and viscosity (mPa*s) were determined before (t0) and after 24 hours (t24) of fermentation. Each parameter was measured in triplicate. Fermentation resulted in a clear increase in total LAB counts in the inoculated samples after 24 h as well as compared with the non-inoculated control, indicating active microbial growth during fermentation (t0 ~ 8.0 log10 CFU/g, t24 ~ 10.0 log10 CFU/g). A consistent decrease in pH values was observed across all fermented samples, reflecting organic acid production by LAB (t0 ~ 6.5, t24 ~ 4.0). In addition, viscosity decreased in all rye cultivars after fermentation (A: t0 ~ 5.6 mPa*s, t24 ~ 4.6 mPa*s; B: t0 ~ 4.4 mPa*s, t24 ~ 2.3 mPa*s; C: t0 ~ 5.5 mPa*s, t24 ~ 2.9 mPa*s). Viscosity values in wheat only changed minimally (t0 ~ 1.7 mPa*s, t24 ~ 1.4 mPa*s). The variables cereal (species and also rye cultivar), bacterial treatment (without inoculation and with the different strains) and cereal*bacterial treatment all significantly influenced absolute pH values, viscosity and LAB counts after 24 hours as well as changes in those parameters from 0-24 hours (ANOVA via linear model, $p < 0.05$). For the cultivars A and C, there was a significant influence of the bacterial strain on change in viscosity for cultivars A, B and C on change in pH values and for cultivars A and B for change in LAB counts from t0 to t24. The pH values and viscosity of non-inoculated control samples changed only slightly after fermentation, highlighting the importance of lactic acid bacteria inoculation in fermentation batches with relatively high dry matter content. These results demonstrate that LAB fermentation significantly alters the physicochemical characteristics of cereal substrates even with high dry matter content. The observed microbial growth, acidification and reduction in viscosity confirm active fermentation and structural modification of the cereal matrix. Such changes may contribute to reducing viscosity-related anti-nutritional effects associated with cereal-based feed ingredients in poultry nutrition making fermentation an interesting technology for sustainable feeding strategies.

Impact of dietary Foeniculum vulgare Mill. on the growth performance of broiler chickens: Insights from a meta-analysis

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Background Increasing global food demand due to population surge warrants nutritionists harnessing every available resource as feedstuff to improve the growth performance of poultry. Poultry farming aims to achieve a reduced feed conversion ratio (FCR); however, this goal is hindered by high feed costs (Alhotan, 2021). Khan et al. (2022) have confirmed the safety of fennel (Foeniculum vulgare Mill.) as a phytogenic ingredient in chicken feed. Authors (Ghonime et al., 2025; İpçak et al., 2024) have reported conflicting data on dietary fennel effects on feed intake (FI), body weight gain (BWG), and FCR, which may be due to differences in strains, dosage, and growth phases. Presently, no systematic attempt has been made to evaluate the impact of these factors on the growth performance of broiler chickens fed diets supplemented with fennel using data from previously published studies. Given the above, this meta-analysis aims to provide data on the effect of diets with or without fennel supplementation on FI, BWG, and FCR in broiler chickens. Methods This study was done by a systematic literature search of studies (2012–2025) reporting the effect of dietary fennel on growth performance in broiler chickens using Scopus, Web of Science, and PubMed databases. A total of 104 studies were identified of which 19 fulfilled the inclusion criteria; thereafter, data were extracted and analysed using OpenMEE. A random effects model was used and presented as a standardised mean difference (SMD) with a 95% confidence interval (CI). Sources of heterogeneity (I_2) were explored by subgroup analysis using continent, sex, strain, form, phase, and dosage. Results Results showed that broiler chickens on dietary fennel had reduced FI (SMD = -0.28; $p < 0.001$; $I_2 = 87\%$) and FCR (SMD = -0.46; $p < 0.039$; $I_2 = 96\%$), while increasing BWG (SMD = 0.2, $p < 0.001$; $I_2 = 94\%$). Subgroup analysis showed that dietary fennel significantly reduced FI in Africa and Asia, mixed-sex groups, Ross, finisher phase, extract form, and at dosages >15 g/kg (SMD ranging from -0.208 to -0.697, $p < 0.05$). Moreover, BWG was significantly improved in Europe, Hubbard and Arbour Acre, and with powder form at 11–15 g/kg showing the greatest effect (SMD = 1.021, $p < 0.001$), while extract form reduced BWG (SMD = -0.659, $p < 0.001$). Dietary fennel reduced FCR in Asia and Europe, Hubbard strain, starter phase, powder form, and at dosages of 11–15 g/kg (SMD = -1.371, $p < 0.001$). Discussion The noted decrease in FI and FCR with improved BWG suggests that dietary fennel promotes feed efficiency. The > 87% I^2 observed was partly explained by subgroup analyses, which showed that the efficacy of fennel inclusion varied depending on continent, strain, growth phase, form, and dosage. Notably, powder improved BWG, while extract reduced it, highlighting the importance of processing methods in determining bioavailability and biological activity of fennel's bioactive compounds. The dose-response relationship was evident for FCR, where 11–15 g/kg produced optimal results, suggesting higher dosages may not confer further benefits or could potentially be detrimental. These findings underscore the need for precision inclusion strategies during the starter phase when feed efficiency is most critical for subsequent performance. Conclusion Fennel inclusion in powder form at 11-15 g/kg significantly improves feed efficiency in broiler chickens, although the substantial I^2 across studies emphasises that optimal benefits are contingent upon careful consideration of broiler strain, growth phase, and geographical production context. Keywords: Fennel; Poultry; Growth performance; Meta-analysis

Effects of dietary Saccharomyces cerevisiae-derived postbiotic on growth performance, intestinal morphology, serum biochemistry, hepatic metabolome and mineral deposition in broiler chickens

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Postbiotics derived from Saccharomyces cerevisiae are promising alternatives to antibiotic growth promoters to improve sustainability in poultry production. This study evaluated the effects of S. cerevisiae-derived postbiotic on growth performance, jejunal morphometric traits, serum biomarkers, liver metabolome profile and mineral deposition in broiler chickens. A total of 96 one-day-old Arbor Acres chicks were randomly assigned to 2 dietary treatments with 4 replicate cages per treatment and 12 birds per cage: a basal control diet (CON) or CON diet supplemented with postbiotic (XPC, Diamond V, USA, 1.0 kg/MT; POST) for 35 d. Body weight and feed intake were recorded weekly per cage to calculate average daily gain (ADG), average daily feed intake (ADFI) and feed conversion ratio (FCR). At d 35, two birds per cage were slaughtered for jejunal histomorphometry, serum biochemistry, mineral analysis of liver and breast muscle, and untargeted LC–MS-based hepatic metabolomics. Data were analyzed by one-way ANOVA with diet as fixed effect (cage as the experimental unit for performance; bird for morphology, serum and mineral traits), and differential liver metabolites were identified using a combination of Student's t-test and OPLS–DA variable importance in projection scores ($P \leq 0.05$, $VIP \geq 1$). Postbiotic improved early growth and overall feed efficiency. POST birds showed greater BW at d 7 ($P = 0.03$) and d 14 ($P = 0.04$), and over d 1–35 had greater ADG ($P = 0.04$), lower ($P = 0.03$) ADFI and improved ($P = 0.03$) FCR (1.47 vs. 1.52) compared with CON. In the jejunum, POST markedly increased villus height (1,907 vs. 1,454 µm; $P < 0.01$), villus height:crypt depth ratio (8.01 vs. 6.53; $P < 0.01$) and villus surface area (1,092 vs. 812 mm²; $P < 0.01$), indicating enhanced absorptive capacity. Serum total protein and calcium were greater ($P \leq 0.05$) in POST birds, whereas alanine aminotransferase, glucose and uric acid were lower ($P \leq 0.05$), suggesting improved protein and mineral status and altered energy and nitrogen metabolism. Postbiotic also shifted mineral distribution, decreasing ($P \leq 0.05$) hepatic Na, Mg and K and increasing ($P \leq 0.05$) breast muscle Mg, K, P, S and Cu. Untargeted liver metabolomics revealed clear separation between POST and CON groups in both positive and negative ion modes, with numerous differential metabolites and enriched pathways, indicating a substantial impact of the postbiotic on hepatic metabolic networks. In conclusion, S. cerevisiae-derived postbiotic improved broiler growth performance, enhanced intestinal morphology, modulated serum biochemistry, shifted minerals deposition and altered the hepatic metabolome, supporting the use of dietary postbiotic as a functional feed additive for a more sustainable broiler production.

Multigenerational effects of in ovo synbiotic and choline supplementation on gut microbiota diversity and composition in broiler chickens

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In ovo delivery of bioactive compounds represents a promising programming strategy to modulate gut microbiota and immunocompetence in the animal. We hypothesized that in ovo injection of synbiotics, with or without choline, exerts cross-generational effects on the structure and composition of the intestinal microbiota in broiler chickens. Fecal samples were collected from young and adult birds across three successive generations (F1–F3) originating from four injection-derived lineages: non-injected control (C), synbiotic single-dose (SYNs), synbiotic repeated-dose (SYNr), and synbiotic with choline single-dose (SYNCHs) and repeated-dose (SYNCHr). Total genomic DNA was extracted using a commercial stool purification kit. The V3–V4 hypervariable region of the 16S rRNA gene was amplified and sequenced on an Illumina NovaSeq 6000 platform (2 × 250 bp). Amplicon sequence variants were resolved via DADA2 within the QIIME2 pipeline and classified against the SILVA 138 reference database. Alpha diversity (Shannon index) was compared using Kruskal–Wallis tests; beta diversity (Bray–Curtis dissimilarity) was assessed by PERMANOVA. Differentially abundant taxa were identified by LEfSe (LDA threshold > 2.0). Shannon alpha diversity increased consistently with age across all generations, reflecting progressive microbiome maturation. Injection-derived effects on alpha diversity were generation-dependent, with significant group differences detected in F2 (young: $p = 0.016$; adult: $p = 0.045$). Beta diversity revealed more pronounced treatment-associated signals. LEfSe identified lineage-specific genus-level biomarkers, including enrichment of fermentation-associated taxa in synbiotic treated lineages. In ovo synbiotic supplementation, in combination with choline, induced cross-generation observed shifts in gut microbiota composition without systematically disrupting global diversity. The findings support the potential of in ovo bioactive interventions as a sustainable strategy for improving gut ecosystem stability and resilience. The research and analysis was funded by National Science Centre grant 2020/37/B/NZ9/00497 and 2019/35/B/NZ9/03186.

Low protein diets in broilers chickens: insight from a holistic evaluation

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Reducing dietary protein has emerged as a promising strategy to improve nitrogen-use efficiency in broiler production and mitigate the environmental impacts associated with poultry protein production. A comprehensive assessment of this approach was carried out, examining its implications for animal response, nitrogen fluxes, economic outcomes and the environmental impacts of broiler production. Three meta-analyses were conducted to identify robust response patterns from the existing literature. A first meta-analysis based on 106 trials confirmed that it was possible to reduce crude protein content without compromising performance, as long as essential amino acid requirements were met. A second meta-analysis highlighted the metabolic consequences of reduced dietary protein, quantifying the associated increases in body lipid accumulation and energy digestibility. A third meta-analysis quantified the effects of reduced protein on nitrogen flow, particularly nitrogen volatilisation and manure production. These findings were complemented by two in vivo studies designed to differentiate protein-level effects from correlated factors such as soybean meal inclusion and dietary electrolyte balance. Results showed that full replacement of soybean meal is achievable but constrained by the protein level, and that reductions in NH_3 emissions arise from both protein and dietary electrolyte balance reduction. In response to limitations of existing model, a new mechanistic model was developed to estimate nitrogen emissions (NH_3, N_2O, NO_x, N_2) across all stages of manure management, from housing to field application. Compared with the European reference model, this new model yields substantially different emission estimates, resulting in either higher or lower environmental impacts depending on the impact category considered. Life-cycle assessments conducted in North American and European contexts demonstrated that reducing dietary protein effectively lowers acidification and terrestrial eutrophication—two impact categories highly sensitive to NH_3 emissions. However, other environmental impacts, including climate change and energy demand, may increase depending on regional context and ingredient sourcing. Economic analyses of costs and benefits further complement this assessment and will be discussed. Overall, this work supports reduced dietary protein as a relevant strategy to address nitrogen-related challenges in poultry production. Future improvements could arise from combining this approach with complementary strategies, such as lowering dietary phosphorus, precision feeding or increasing the use of low impact raw materials.

Comparative effects of a multi-carbohydrase and single xylanase supplementation on broiler performance and environmental impacts

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Exogenous carbohydrases can alleviate the anti-nutritional effects of non-starch polysaccharides in poultry diets, improving nutrient availability and feed efficiency. This allows lowering dietary nutrient density without compromising performance, while reducing environmental impacts. This study evaluated the effects of supplementing nutrient-reduced corn-wheat-soybean meal diets with either a multi-carbohydrase complex (MCC) or a single xylanase (SX) on broiler performance and environmental impacts quantified by life cycle assessment (LCA). A total of 1,480 day-old male Ross 308 broilers were randomly allocated to four dietary treatments with 10 replicate pens per treatment and 37 birds per pen. Dietary treatments included: (1) a positive control (PC) formulated to meet animal requirement; (2) a negative control (NC) formulated with a 3% reduction in apparent metabolisable energy and digestible amino acids relative to PC; (3) the NC supplemented with MCC (50 g/ton of feed); and (4) the NC supplemented with SX (70 g/ton of feed). Performance parameters (feed intake, body weight (BW), BW gain (BWG), feed conversion ratio (FCR), and mortality) were evaluated over a 35-day experimental period. Data were analysed by ANOVA (GLM) with significance declared at $P < 0.05$, and treatment means separated using the Tukey test. A cradle-to-feed mill and farm-gate LCA was conducted using the feed, performance, and default European broiler farm data. Feed ingredient impacts were obtained from GFLI v2.2 (2024), with origins reflecting average Polish and European production and imports. Sixteen environmental footprint (EF) impact categories were assessed in accordance with PEFCR and FAO LEAP (2020) guidance. Results are expressed per 1 kg feed and 1 kg broiler liveweight (LW) as functional units. For the overall trial (0–35 d), feed intake, BW and BWG were similar ($P > 0.05$) among treatments. The NC diet increased FCR compared to PC (1.582 vs. 1.493; $P < 0.05$). The MCC supplementation improved FCR relative to NC (1.502 vs. 1.582; $P < 0.05$) and restored FCR to PC levels (1.502 vs. 1.493; $P > 0.05$). However, the SX diet resulted in an FCR comparable to NC (1.574 vs. 1.582; $P > 0.05$) and failed to restore FCR ($P < 0.05$) to PC levels. Based on 1 kg feed at the feedmill gate, the NC formulations (starter, grower and finisher) reduced all 16 EF impact categories compared to PC. The largest reductions occurred for total climate change (−5% to −7%; -0.06 to -0.08 kg CO2eq/kg feed), freshwater ecotoxicity (−5% to −6%; -5.83 to -7.81 CTUe/kg feed), land use (−3% to −4%; -5.04 to – 6.70 Pt/kg feed), and water use (−8% to −11%; -0.04 to -0.05 m3 depriv./kg feed). For climate change impacts, the primary feed-related hotspots were soybean meal and oil from European imports. Based on 1 kg of broiler LW, the MCC diet reduced 14 of 16 EF impact categories compared to PC, with notable decreases in total climate change (-4 %; -0.09 kg CO2eq/kg LW), climate change-land use and land use change (-10%; -0.09 kg CO2eq/kg LW), land use (-3%; -7.39 Pt/ kg LW), water use (-7%; -0.06 m3 depriv./kg LW) and freshwater ecotoxicity (-4%; -9.33 CTUe/kg LW). However, NC and SX supplementation reduced only 2 of 16 EF impact categories relative to PC (-6% and -5% (-0.05 and -0.04 kg CO2eq/kg LW) for climate change-land use and land use change; -3% and -2% (-0.03 and -0.02 m3 depriv./kg LW) for water use, respectively). Hence, SX supplementation did not reduce the overall EF mainly due to impaired broiler performance. In contrast, MCC supplementation in nutrient-reduced diets mitigated the environmental impacts of broiler production without compromising performance.

Multi-carbohydrase and bacterial 6-phytase co-supplementation improves performance, nutrient digestibility and feed cost efficiency in broilers fed nutrient-reduced corn-soybean meal diets

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Exogenous carbohydrases and phytase are widely used in broiler diets to improve nutrient availability by degrading non-starch polysaccharides and phytate, thereby supporting dietary nutrient reductions without compromising performance. However, evidence supporting their efficacy when co-supplemented within multi-nutrient matrix reduction strategies remains limited. This study evaluated the effects of multi-carbohydrase and bacterial 6-phytase co-supplementation on performance, nutrient digestibility, and feed cost efficiency in broilers fed corn-soybean meal diets reduced in energy, amino acids (AA), Ca and P. A total of 756 day-old male Cobb 500 chicks were randomly assigned to three dietary treatments with 12 replicate pens of 21 birds each. Treatments included: (1) a positive control (PC; standard diet) formulated to meet animal requirements; (2) a negative control (NC) diet reduced by -150 kcal/kg apparent metabolisable energy (AME), -5% digestible AA and -0.19% Ca and P relative to PC; and (3) an enzyme (ENZ) diet consisting of NC supplemented with 50 g/t feed of a multi-carbohydrase complex (Rovabio® Advance, Adisseo, France) and 1000 FTU/kg feed of bacterial 6-phytase (Rovabio® PhyPlus, Adisseo, France). Feed intake, body weight (BW), BW gain (BWG), feed conversion ratio (FCR) and mortality were measured across starter (0–14 d), grower (14–28 d), and finisher (28–35 d) phases. The European Production Efficiency Factor (EPEF) and feed cost efficiency (feed cost €/kg BWG) were calculated for 0–35 d. Excreta were collected on days 21–24 to determine apparent dry matter (DM), gross energy, protein, starch and fat digestibility, and for the calculation of AME. Data were analysed as a randomised complete block design with pen as the experimental unit, using PROC MIXED in SAS. Significance was declared when $P < 0.05$, and treatment means were compared using the Tukey-Kramer test. Across phases, PC birds showed higher ($P < 0.001$) feed intake, BW, and BWG, and lower ($P < 0.001$) FCR than NC birds. The ENZ diet restored BW and BWG to similar levels ($P > 0.05$) as the PC in all phases. Over 0–35 d, the PC diet increased ($P < 0.001$) feed intake (3.18 vs. 2.57), final BW (2.51 vs. 1.88) and BWG (2.47 vs. 1.84), and lowered ($P < 0.001$) FCR (1.375 vs. 1.513) relative to the NC diet, resulting in higher EPEF (494 vs. 334). The ENZ diet improved ($P < 0.05$) feed intake (3.31 vs. 2.57 kg), final BW (2.47 vs. 1.88 kg), BWG (2.44 vs. 1.84 kg), FCR (1.442 vs. 1.513) and EPEF (484 vs. 334) compared with NC. Similarly, the ENZ diet restored final BW, BWG, and EPEF to levels comparable ($P > 0.05$) to the PC. Performance improvements in ENZ birds were supported by higher digestibility ($P < 0.001$) of DM (+6.2%), protein (+12.2%) and energy (+6.6%), resulting in increased AME (+192 kcal/kg) compared to NC. Apparent digestibility did not differ between ENZ and PC diets ($P > 0.05$), except that ENZ-fed birds exhibited higher ($P < 0.05$) digestibility of DM (+4.2%) and energy (+2.4%). The ENZ diet improved ($P < 0.01$) feed cost efficiency by 3.5% (-0.0126 €/kg BWG) and 2.4% (-0.0085 €/kg BWG) compared to PC and NC diets, respectively. Relative to the PC diet, the ENZ diet could translate to an estimated saving of €31,463 per million birds (2.5 kg target BW). In conclusion, co-supplementation of a multi-carbohydrase complex and bacterial 6-phytase in multi-nutrient-reduced corn-soybean meal diets effectively maintained broiler performance and nutrient digestibility at levels comparable to the standard diet, while significantly reducing feed cost.

Phytogenic dietary inclusion level modulates key performance indicators and meat quality biomarkers in heat-challenged broilers

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Heat stress represents one of the most detrimental stressors affecting poultry production worldwide. This study evaluated the dose-dependent effects of a dietary phytogenic blend (PB) on broiler growth performance and meat quality following cyclic heat stress during the finisher phase. In addition to growth performance responses, meat antioxidant status and key molecular biomarkers relevant for antioxidant (Nuclear factor erythroid 2-related factor 2; Nrf2) response were assessed. One day-old, Ross 308 (n=490) broilers were allocated to 5 dietary treatments, with 7 replicates of 14 chickens each, for a 40-d feeding trial. Experimental treatments received corn-soybean meal basal diets with no PB (CON) or supplementation with PB at 500 (PB500), 1000 (PB1000), 1500 (PB1500) and 2000 mg/kg diet (PB2000), respectively. The PB had olive oil polyphenols, carvacrol and thymol among its main bioactive components (nuPhoria®; Nuevo SA, Greece). Birds were reared under thermoneutral conditions for 35 days and then exposed to cyclic heat stress (32 ± 1 °C; RH 55 ± 5%) for 7 h/day during days 36–40. Growth performance was recorded per growth phase and results reported as overall. At day 40, breast meat samples were collected for antioxidant capacity determination, and gene expression analysis. Data were analyzed using ANOVA with means compared by Tukey's honest significant difference test and statistical significance was determined at $P \leq 0.05$. Polynomial contrasts tested the linear and quadratic effects of PB inclusion levels. Results revealed that PB inclusion improved ($P \leq 0.05$) overall FCR, protein efficiency ratio and energy efficiency ratio in PB1000 and PB1500, compared to CON. Moreover, PB supplementation improved breast oxidative stability, as reflected by reduced malondialdehyde ($P < 0.001$) and protein carbonyl concentrations ($P = 0.048$), with the most pronounced responses observed at 1000–1500 mg/kg diet. In addition, the breast total antioxidant capacity increased with PB inclusion ($P < 0.001$), reaching the highest levels at 1500 and 2000 mg/kg, compared to the CON. At the molecular level, PB antioxidant capacity was evidenced via beneficial changes ($P \leq 0.05$) seen for the majority of the Nrf2-pathway genes assessed in the breast. In conclusion, dietary PB improved broiler performance and enhanced breast meat oxidative stability under cyclic heat challenge conditions. The improved breast quality was further supported by beneficial modulation of critical antioxidant genes. Considering collectively the overall performance and meat quality biomarkers, PB inclusion levels at 1000 and 1500 mg /kg diet proved beneficial for heat-challenged broilers.

Effect of black soldier fly larvae oil and feed withdrawal on productive performance in broiler chicken subjected to cyclic heat stress during the final growth period

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Medium-chain fatty acids (MCFAs) present in black soldier fly larvae oil (BSFO) possess antimicrobial, and anti-inflammatory properties, and provide rapidly available energy, which might support the physiological response and intestinal health of poultry under heat stress conditions. In addition, controlled feed withdrawal during peak heat periods has been proposed as a strategy to improve thermoregulation by preventing the postprandial heat increment from coinciding with maximal ambient temperatures. The aim of this study was to evaluate whether the inclusion of BSFO rich in MCFAs, combined with a 6-hour feed withdrawal, could mitigate the negative effects of cyclic heat stress during the final growth period on broiler performance. It is hypothesized that combining a lipid source rich in MCFAs with feed withdrawal during peak heat periods may mitigate thermogenic load and support intestinal health improving productive performance. For this purpose, 540 one-day-old Cobb 500 broilers were randomly allocated to 54 pens and fed the same commercial diets from 1 to 20 days of age. Broilers were then divided into 6 groups (9 replicates per treatment; 10 birds per pen) following a completely randomized design. Two experimental groups were kept under thermoneutral conditions and fed isoenergetic (3,079 kcal/kg) and isoproteic (19% CP) corn–soybean diets supplemented with 5.5% soybean oil (SBO) or BSFO from 22 to 35 days of age. The other four groups were also housed under thermoneutral conditions until day 29, then exposed to cyclic heat stress (temperature-humidity index 80) for 6 hours daily from days 30 to 35. These birds received SBO or BSFO diets, and two of the groups additionally underwent a 6-hour feed withdrawal during the peak heat-stress period. Main effects stress (thermoneutral or cyclic heat stress), fat source (SBO or BSFO), feed withdrawal and their interactions were analysed with orthogonal contrasts using the General Linear Model procedure of SAS. Average daily feed intake (ADFI), average daily gain (ADG), and feed conversion ratio (FCR) were recorded at 29 and 35 days of age. No significant differences in performance parameters were observed between 21 and 29 days of age in broilers fed SBO or BSFO ($P > 0.05$). During the period from 30 to 35 days, heat-stressed birds showed lower ADG ($P < 0.001$) and ADFI ($P = 0.003$), as well as higher FCR ($P = 0.002$), compared to birds maintained under thermoneutral conditions. No significant effects of fat source or feed withdrawal under heat stress were found ($P > 0.05$), and no interactions among the evaluated factors were observed. In conclusion, cyclic heat stress applied during the final growth period negatively affected broiler performance, reducing ADG and ADFI and increasing FCR. Neither the BSFO nor the feed withdrawal improved the productive parameters during heat stress, showing similar results to ad libitum feeding and SBO. This research was supported by grant PID2023-149145OB-I00 funded by MICIU/AEI/10.13039/501100011033 and ERDF/EU.

Effect of Marine mineral complex on skeletal mineral deposition and liver health in aging laying hens

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Modern agricultural practices require an extended laying cycle for layers to meet with efficiency and supply demands of a growing population. Through management, genetic and nutritional practices, the laying period can be extended up to 100 weeks. However, during extended lay the birds are faced with the problem of declining bone quality due to deterioration in the absorption, retention and metabolic supply of minerals such as calcium (Ca) and phosphorus (P). This often results in stress, inflammation, increased mortality and consequently reduced flock laying performance and eggshell quality. In this study, we investigated the effect of a marine mineral complex (MMC) in several particle sizes and doses of compared with feed-grade limestone as Ca source on bone quality, bone mineral deposition, and liver health. The study involved feeding 448, forty week old Hy-line brown layers, grouped into 76-77 birds per treatment group, with the following treatments: a) control diet with coarse and fine limestone with 3.8%w/w total Ca in the diet, b) coarse MMC (at 0.25%), partially replacing coarse limestone c) coarse MMC (at 0.5%), d) commercial MMC (0.5%) partially replacing fine limestone, e) fine MMC (0.25%), and fine MMC (0.5%) partially replacing fine limestone. At the end of the trial, bone and liver samples were collected from 22 hens per treatment group for analysis. The data showed that MMC significantly ($P<0.05$) affected mineral deposition in the tibia and toe bones with inclusion of fine particle MMC leading to higher mineral deposition (Ca increase by 3.1% and Sr by 20%). Keel fracture (reduced by 23%) and curvature scores (reduced by 14%) were numerically lower in MMC treatment group, but differences were not significant ($p>0.05$). Fatty liver scores were significantly ($P<0.001$) reduced by MMC versus limestone control (1.35 v 2.32, respectively) with an effect of dose and MMC particle size detected. High dose and large particle size MMC inclusion provided the biggest reduction in fatty liver scores. Overall, inclusion of MMC in layer diets are shown to improve bone mineral deposition and liver health, making it an interesting product to use in layers and especially in the case of extended lay.

Assessing Selenium Source Bioavailability via Excretion Patterns in Broiler Feces

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Selenium (Se) is an essential micronutrient involved in antioxidant defense and metabolic functions, yet its homeostasis requires tight regulation to avoid toxicity. In poultry, urinary and fecal routes merge in the cloaca, making fecal Se excretion a potential indicator of dietary Se utilization. This study aimed to evaluate whether fecal Se output can serve as a practical biomarker of Se source bioavailability in broilers. Four trials were conducted using ROSS 308 broilers fed iso-selenium diets (0.5 ppm total Se) containing either inorganic sodium selenite (SS) or selenium sources, including Se-enriched yeast (S. cerevisiae R397, SC R397). Birds were reared under standard conditions, and intestinal fecal samples were collected at day 14 (Trials 1 to 4) and day 32 (Trial 1). Selenium concentration in feces was determined after lyophilization, and excretion ratios (Se feces/Se intake) were calculated to assess retention efficiency. Results consistently showed that organic Se sources, particularly Se-enriched yeast, were less excreted than mineral forms. Despite increased intake levels, organic Se did not proportionally raise fecal Se output, suggesting higher tissue retention. Among all tested sources, SC R397 exhibited the lowest excretion ratio and the highest Se deposition in tibia (1,937 µg/kg FM), confirming superior bioavailability compared to SS (1,557 µg/kg FM). Conversely, sodium selenite displayed the highest fecal Se concentrations (up to 1,660 µg/kg DM) and ratios exceeding 46%, while SC R397 remained below 40%. These findings align with previous reports (Barbé et al., 2017; Leukert et al., 2026) highlighting the efficiency of yeast-based Se in improving Se status and reducing waste. In conclusion, fecal Se analysis offers a simple, non-invasive approach to compare Se sources in poultry nutrition. Lower fecal excretion reflects better absorption and retention, making this parameter a valuable tool for assessing bioavailability. Further research should integrate statistical modeling and explore correlations with performance and oxidative stress markers to strengthen its predictive value.

Embryonic stage-dependent effect of in ovo synbiotic administration at day 12 vs. day 18-implications for immunocompetence conditioning

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In ovo administration of microbial bioactives has the potential to prime the developing immune system, enhance mucosal barrier integrity, and stimulate gastrointestinal tract function prior to hatch — effects with downstream consequences for performance, disease resilience, and food safety. The embryonic stage at the time of intervention is a critical determinant of these outcomes, yet comparative data on ED12 versus ED18 administration remain limited. This study aimed to evaluate the developmental stage-dependent effects of in ovo synbiotic injection administered at embryonic day (ED) 12 versus ED18 on post-hatch broiler growth, serological responses to commercial vaccination, and jejunal histomorphometric parameters in broiler chickens. Fertilized Ross 308 broiler eggs from a 45-week-old flock were incubated and allocated to experimental treatment groups receiving in ovo injection at ED12 (manual injection into the air chamber; 200 µl total volume: 2 µl 1% prebiotic extract + 188 µl physiological saline + 10 µl Lactobacillus rhamnosus H25 at 1×10^4 CFU/egg) or ED18 (50 µl total volume: 25 µl 1% prebiotic solution + 2.5 µl probiotic suspension at 1×10^3 CFU/egg + 22.5 µl live attenuated IBD vaccine), alongside a non-treated negative control group. Birds were reared under standardised commercial conditions. In ovo synbiotic injection at ED12 (group B: SYN-12ED) produced higher body weight gain, becoming most pronounced during the terminal phase of the rearing period. Serological analyses demonstrated the elevated post-vaccination antibody titres in synbiotic-treated groups relative to controls, suggesting enhanced B-cell-mediated humoral activation facilitated by synbiotic-induced immune conditioning. The findings demonstrate that the embryonic time point of synbiotic delivery critically determines the direction and magnitude of post-hatch outcomes. This study validates in ovo administration of microbial bioactives and natural immunomodulators as an evidence-based tool for early-life immunocompetence conditioning, supporting the One Health imperative for resilient production systems. The research was supported by the collaborative Ovobiom project grant number 2019/35/B/NZ9/03186, National Science Centre in Poland.

Lipid structure and energy utilization in high-performance broiler diets: impacts on performance, carcass traits and oxidative status

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Dietary lipids are essential components of energy-dense broiler diets and play a central role in nutrient digestibility, metabolic energy supply and carcass composition. Fatty acid chain length and saturation influence digestion kinetics, absorption pathways and post-absorptive metabolism. However, the extent to which structurally different lipid sources affect nutrient partitioning under modern high-performance conditions remains unclear. Therefore the present study was conducted to evaluate the effect of different fats differing in fatty acid profile and physical structure on growth performance, carcass characteristics and physiological parameters in broiler chicken. A total of 198 one-day-old male Ross 308 broilers were allocated to three dietary treatments (6 replicates; 11 birds per pen). Following a common starter phase (d1–10; 208 g/kg CP, 79 g/kg CL, 12,0 MJ AMEN/kg), birds received isoenergetic grower- (d11–23; 216 g/kg CP, 72 g/kg CL) and finisher diets (d25–35; 210 g/kg CP, 75 g/kg CL) containing either soybean oil (feed group 1, FG 1), a spray-cooled palm oil-based fat powder enriched with lecithin (FG 2; BEWI®-SPRAY 99L), or a mixture of short- and medium-chain fatty acids incorporated in a rapeseed oil-based matrix (FG 3; BEWI®-FATRIX SynerG+). Lipid inclusion ranged from 3.4 to 3.8 %. Performance parameters were recorded until d35. Carcass traits were determined post-slaughter. Plasma thiobarbituric acid-reactive substances (TBARS), ferric reducing ability of plasma (FRAP) and advanced oxidation protein products (AOPP) were analyzed to assess oxidative status. Data were evaluated using one-way ANOVA with Tukey-Kramer adjustment ($p \leq 0.05$). Growth performance was high across all treatments. Final body weight averaged 3,040 g and feed conversion ratio 1.34, with no significant differences among groups ($p > 0.05$). In contrast, carcass parameters were affected by lipid source. Dressing percentage was highest in birds from FG 3 ($p \leq 0.05$). Both alternative treatments with spray-cooled fat powder increased absolute breast meat yield compared with soybean oil (675 g (FG 1) vs. 746 g and 747 g (FG 1 and FG 2, respectively), and lecithin inclusion improved relative breast meat proportion ($p \leq 0.05$). No treatment effects were observed for TBARS, FRAP or AOPP ($p > 0.05$), indicating that substitution of soybean oil did not induce measurable systemic oxidative stress. Although the literature describes increased digestibility of short- and medium-chain fatty acids compared to long-chain fatty acids, this study found no significant differences in performance parameters. The absence suggests comparable metabolizable energy utilization across treatments when diets are formulated isoenergetically. However, differences in carcass yield indicate that lipid structure may influence nutrient partitioning and muscle deposition independently of overall growth rate. Lecithin supplementation may have supported emulsification and intestinal lipid utilization. In conclusion, structurally distinct lipid sources such as spray-cooled fat powders can replace soybean oil in high-energy broiler diets without impairing performance or oxidative stability, while influencing carcass composition. These findings underline the importance of lipid structure in poultry nutrition and support strategic fat source selection as a tool to optimize nutrient efficiency and product value.

Enhancing broiler chickens' performance through synergistic citrus extract prebiotic and probiotic supplementation

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In recent years, probiotics have gained increasing attention for their potential to beneficially modulate the gut microbiota and improve intestinal function, highlighting their relevance in the prevention and management of gastrointestinal and metabolic disorders. However, their efficacy may be limited by microbial competition, variability in gut ecology, and various stresses related to livestock farming. Synergistic interactions between a targeted probiotic strain and a complementary prebiotic substrate may enhance intestinal health more effectively than the probiotic alone, leading to better animal performance. Therefore, this study aimed to assess the effects of a symbiotic combination of a probiotic and a citrus extract prebiotic on broiler chickens' performance, compared to the probiotic alone. In an experimental station in Algeria, 540 broiler chickens (Arbor Acres, Aviagen) were randomly divided into 3 groups: a control group (CTL group) fed with a standard diet;a probiotic group (PRO Group) supplemented with the recommended dose by the producer of a commercial probiotic (200 ppm), added to water, and fed with the standard diet;a symbiotic group (SYM Group) fed with a standard diet supplemented with 80% of the recommended dose of a citrus extract prebiotic (Nor-Spice AB®, Nor-Feed SAS, 200 ppm) and 50% of the recommended dose of the same probiotic used in the PRO group (100 ppm).Supplementation occurs from day 1 to day 46. Chickens were put under a thermic stress at 30°C for 4 hours from day 26 to day 30. Growth performance (weight, feed intake, and mortality) of each group were weekly recorded. The Feed Conversion Ratio (FCR) and the average daily gain (ADG) were also calculated. Results showed that chickens from the SYM group have a higher weight (2647.97) than chickens from the PRO group (2608.57) and the CTL group (2504.79, $p < 0.05$, ANOVA). The ADG was also significantly higher in the SYM group (68.08 g/day) compared to the pro group (66.86 g/day) and the CTL group (63.31 g/day, $p < 0.05$, ANOVA). Regarding feed intake, broiler chickens from PRO group had a higher overall feed intake (5,214 g/chicken) compared to the SYM group (5,171 g/chicken) and the CTL group (5,001 g/chicken). As a consequence, the FCR from the SYM group was lower (2.11) than the FCR from CTL (2.20) and PRO groups (2.18, $p < 0.05$, ANOVA). No difference was observed between broiler chickens' mortality from the 3 groups ($p > 0.05$, ANOVA). These results show that the combined administration of citrus extract and probiotics exerts a greater positive effect on broiler growth performance than the probiotic alone. Such a symbiotic strategy represents a promising nutritional alternative to antibiotic growth promoters for sustaining growth performance in poultry production systems.

Evaluation of protected sodium butyrate supplementation to optimize gut health biomarkers in broiler chickens

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Butyrate is recognized to be a potent modulator of gastrointestinal health, exerting its effects by enhancing intestinal epithelial integrity, supporting the immune system, and promoting a beneficial shift in the commensal microbiota. This study aimed to evaluate the effects of a protected sodium salt of butyric acid on gut health biomarkers in broilers. A total of 300 one-day-old broiler chicks were randomly assigned to 2 dietary treatments with 10 replicate pens per treatment and 15 birds per pen. The treatments included a control group fed a standard diet (CTR) and a treatment group fed the CTR diet supplemented with 1 kg/t of sodium butyrate protected by sodium salts of palm fatty acid distillates (GR, Gustor N'RGY, 70% sodium butyrate, Norel Animal Nutrition). Chicks were fed pelleted diets for 35 days. At 35 days, blood samples were collected for immunoglobulin (IgY and IgA), cytokines (TNFα and IL10), and antioxidant [Total Antioxidant Capacity (TAC), superoxide dismutase (SOD) and glutathione (GSH)] analysis. Duodenal and ileal tissues were sampled and analyzed for histology (villi height and crypt depth), and mRNA expression of tight junction proteins (OCL, MUC-2, and ZO-1). Cecal content was collected at 35 days and analyzed for bacterial count of Coliform and Clostridium. Data were analyzed using one-way ANOVA in RStudio. Results of blood immunoglobulin levels were the highest in GR compared to CTR for IgY (424 vs. 328 ng/ml, $P < 0.05$) and were similar for IgA (530 vs. 474 ng/ml, $P > 0.05$). Cytokine concentrations showed the lowest TNFα concentration in GR compared to CTR (351 vs. 503 Pg/mL, $P < 0.05$), and similar IL10 concentrations between treatments (503 vs. 439 Pg/mL, $P > 0.05$). Blood antioxidant analysis showed the highest values in GR compared to CTR for TAC (5.64 vs. 4.54 ng/ml, $P < 0.05$) and GSH (150 vs. 114 ng/ml, $P < 0.05$), but no difference for SOD (134 vs. 132 U/ml, $P > 0.05$). Duodenal villi height and the ratio of villi height to crypt depth were the highest in GR compared to CTR (1072 vs. 1037 µm and 6.46 vs. 6.05, $P < 0.05$, respectively). Crypt depth at the duodenum level wasn't affected by treatment. At the ileal level, villi height was also higher in GR compared to CTR (485 vs. 472 µm, $P < 0.05$). However, crypt depth and the ratio of villi height to crypt depth were not different between treatments ($P > 0.05$). The GR treatment also improved tight junction expression for OCL (2.54 vs. 1.00), ZO-1 (1.87 vs. 1.00), and MUC-2 (2.01 vs. 1.00) compared to the CTR ($P < 0.05$). For microbial count analysis, lower Coliform and Clostridial counts were observed in GR compared to CTR (46% and 65% lower, $P < 0.05$, respectively). In summary, protected sodium butyrate was effective in improving gut health biomarkers, indicating better intestinal homeostasis. This enhanced resilience is critical in modern broiler production, providing the physiological foundation necessary to maintain high performance and efficiency.

Effects of Dietary Energy Levels During the Growing and Early-Laying Periods on Late-Laying Performance, Egg Quality, and Organ Characteristics in Laying Hens

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Achieving a 100-week laying cycle in commercial layers necessitates nutritional approaches that preserve productivity and metabolic health in aging hens. Accordingly, this study investigated the impact of lowering dietary metabolizable energy (ME) levels during the growing and early laying periods on hepatic condition and productive performance during the late laying period. A total of 360 Hy-Line Brown pullets (3 weeks of age; initial body weight 205.8 g; uniformity 76.6%) were assigned to four diets differing in ME density [high (H), control (CON), low (L), and very low (VL)]. The control (CON) diet was formulated according to the breeder manual recommendations, and the other treatments were adjusted relative to CON as follows: H, CON +100; L, CON −100; and VL, CON −200 kcal/kg. Diets were fed from 3 to 18 weeks of age (growing period) and from onset of lay to 36 weeks (early laying period), after which all birds received the same commercial layer diet. Late-laying performance (46–60 weeks of age), egg quality, and organ characteristics were evaluated. At 60 weeks of age, selected hens were euthanized for measurements of abdominal fat, liver traits (weight, fat content, malondialdehyde (MDA)), and ovarian characteristics. Data were analyzed by one-way ANOVA using the GLM procedure of SAS, with means separated by Duncan's test ($P < 0.05$). Late-laying hen-day egg production, egg weight, egg mass, feed intake, feed conversion ratio, and standard egg quality traits (shell strength and thickness, yolk color, albumen height, Haugh units) did not differ among treatments. Abdominal fat and oviduct weight were also unaffected. In contrast, VL hens showed lower liver weight than H hens, and hepatic fat content decreased significantly from H to L and VL. Liver MDA concentration declined progressively with decreasing dietary ME and was lowest in VL hens. Moreover, L and VL hens showed higher weights of the largest and second-largest preovulatory follicles (F1, F2), and VL hens had more small yellow follicles (5–10 mm) but fewer large yellow follicles (>10 mm) than H and CON. These findings indicate that reducing dietary energy density during the growing and early laying periods can mitigate hepatic fat accumulation and oxidative stress in late-laying hens without compromising productivity, thereby potentially improving metabolic robustness for extended laying cycles. However, the slight numerical increase in feed conversion ratio observed in lower-energy diets warrants further investigation to determine its biological relevance and whether these early nutritional effects persist through 100 weeks of age.

Climate protection and food security: antagonists or synergists in grassland-based production?

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The call for mitigation of enteric methane has a tendency to shift cattle production systems towards intensification, because higher concentrate-to-forage ratios may reduce emissions per kg of milk or meat. Furthermore, precision livestock feeding (PLF), aiming at maximising nutrient efficiency also requires tailored diets, with considerable amounts of concentrated components offered as mixtures which avoid selective behaviour of the cows; thus PLF solutions are often barn-based and intensive. Against these practises, pasture- and grassland-based production systems are facing challenges regarding justification of higher product-related methane emissions, apparently low resource efficiency and high land-requirements. However, with a feed-food competition perspective, land-use and edible food efficiency can comprise a clear advantage for grassland-based over intensive systems. Especially in regions and systems, which possess or require larger shares of grassland (permanent pastures, or, in organic systems, crop-rotation grass-clover leys), their utilisation can provide a significant contribution to animal sourced proteins outside the feed-food competition. Furthermore, grassland landscapes bring along several relevant ecosystem services, such as biodversity, carbon stocks and animal welfare aspects. Crucial questions resulting are, (1) if carbon footprints of grassland-based vs. intensive cattle production systems can be fairly compared, and on which level of system boundaries this should be done, (2) which measures of methane mitigation are practicable for grassland-based systems, and (3) how can costs and benefits of different targets be balanced in the optional actions. This lecture will provide an overview of quantifications to compare different production systems and raise the question, how different targets such as low carbon footprint vs. food security can be prioritzed, balanced or joined. Not the least, various emission mitigation approaches in grassland-based systems will be discussed, in order to reconcile multiple targets of sustainable livestock agriculture.

Enhancing MIR-based methane prediction through systematic integration of cow- and farm-level information

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The quantification of methane emissions is essential for sustainable dairy production, yet direct measurements remain costly and difficult to scale. Mid-infrared (MIR) milk spectra provide a practical and widely available data source in Austria. This study evaluated whether MIR-based methane prediction can be improved through structured data integration and whether increasing information depth requires more complex modelling approaches. The dataset comprised 12 Austrian dairy farms with 444 cows and 574 repeated observations (cow × period). Methane emissions were defined as 6-week mean CH_4 production (g/day), aligned with a representative MIR sampling week. The value of contextual information was assessed through a hierarchical data integration strategy. Starting from a MIR-only baseline, additional data blocks were sequentially incorporated: routinely available cow-level variables from dairy herd improvement (DHI) records (e.g., parity, milk yield), non-routinely recorded cow-level traits requiring additional measurements (e.g., body weight, feed intake), herd-level indicators summarizing long-term performance (e.g., longevity, disease incidence, annual herd milk yield), and farm management characteristics (e.g., pasture access, diet composition). At each integration stage, four modelling approaches were evaluated — Partial Least Squares (PLS), Random Forest, XGBoost, and Support Vector Machines — enabling systematic assessment of whether increasing information depth necessitates more complex models. Model performance was assessed under two complementary validation settings reflecting distinct deployment objectives. Within-farm predictive performance was quantified using animal-grouped cross-validation, preserving intra-animal dependencies and evaluating prediction for new animals within previously observed farms. Structural transportability was assessed using leave-one-farm-out validation, where each farm was excluded during training and used exclusively for testing to evaluate generalization across heterogeneous production systems. Results from the within-farm validation framework showed that prediction accuracy improved consistently with increasing information depth. Using the full information set, PLS achieved the best performance (RMSE = 48.12 g/day, r = 0.73, R^2 = 0.54), closely followed by XGBoost (RMSE = 49.00 g/day, r = 0.72, R^2 = 0.52). These results indicate that linear modelling is sufficient to capture most of the additional predictive signal introduced by structured data integration. Under leave-one-farm-out validation, overall performance decreased, reflecting substantial between-farm heterogeneity. PLS again performed best but with clearly reduced accuracy (RMSE = 56.65 g/day, r = 0.61, R^2 = 0.39), indicating limited structural transferability across farms. More flexible machine learning approaches did not provide consistent improvements under this setting. In conclusion, structured data integration is the primary driver of improved MIR-based methane prediction, whereas model complexity remains of secondary importance in this data regime. However, cross-farm generalization remains a key limitation and requires further methodological and data-related research.

Methane emission and feeding behavior of dairy cows grazing versus indoor feeding with silage

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Grazing cattle may have lower methane emission than cattle fed grass-clover as silage indoors. This study aimed to quantify enteric methane emission and eating and rumination time of Danish Holstein dairy cows grazing perennial ryegrass and white clover pasture compared to dairy cows fed grass-clover silage indoors. The experiment covered 2 weeks of adaptation and 9 experimental weeks (May 6th to July 7th, 2024). Three treatments were allocated to 45 lactating dairy cows (15 cows/treatment) in a continuous design: 1) indoor feeding with a spring growth (first cut) grass-clover silage (GCS), 2) grazing during day and night (DAY+NIGHT), and 3) grazing during the day (ca. 8 h) with indoor feeding of grass-clover silage during the night (DAY+GCS). Methane emission was measured with the GreenFeed system (C-Lock Inc., USA) using 2 units indoors and 2 units placed in huts on pasture. Feeding behavior was measured with RumiWatch (ITIN+HOCH GmbH, Switzerland) over 6 days repeated over 3 weeks for a subset of cows and only for the GCS (6 cows) and the DAY+NIGHT treatments (6 cows). Concentrate was provided in GreenFeed (up to 2.4 kg dry matter/d; DM) and in the milking parlor (2.7 kg DM/d). The grazing system was strip grazing with rotations over paddocks. Pre- and post-grazing herbage mass per paddock was measured with Grasshopper (True North Technologies, Ireland). The pre-grazing herbage mass was on average 1259 kg DM/ha, and the pre- and post-grazing sward heights were on average 99 and 68 mm, respectively. Total dry matter intake (DMI) was the sum of DMI of concentrate, grass-clover silage and/or pasture. Pasture DMI was calculated using energy requirements for maintenance, milk production, gestation, growth, mobilization, and deposition, and energy intakes with concentrate and silage. Preliminary data show that DAY+GCS had the highest total DMI (20.9 kg/d) and energy-corrected milk (ECM) yield (31.7 kg/d), while DAY+NIGHT had the lowest total DMI (19.5 kg/d) and ECM yield (28.8 kg/d) with GCS being intermediate in total DMI (19.9 kg/d; P=0.01) and ECM yield (29.9 kg/d; P<0.001). A significant interaction between treatment and week (P<0.001) was observed for both variables. Weekly GreenFeed visits per cow were on average 26, 15, and 23 visits/week for GCS, DAY+NIGHT and DAY+GCS treatments, respectively. Methane emission was lower for DAY+NIGHT (311 g/d and 11.2 g/kg ECM) compared to GCS (449 g/d and 15.1 g/kg ECM) with DAY+GCS being lower (409 g/d and 13.0 g/kg ECM; P<0.001 for both) than GCS. Compared to GCS, methane (g/kg ECM) was 25.8 and 14.2% lower for the DAY+NIGHT and DAY+GCS treatments, respectively. No significant interaction between treatment and week was observed for both methane variables (P>0.05). For DAY+NIGHT, total eating time (620 min/d) and per unit DMI (29.9 min/kg DMI) were higher than for GCS (446 min/d and 20.7 min/kg DMI; P<0.001), whereas total rumination time and per unit of DMI were lower for DAY+NIGHT (450 min/d and 21.8 min/kg DMI) than GCS (543 min/d and 25.3 min/kg DMI; P=0.001 and 0.02, respectively). Both eating time variables showed a significant interaction between treatment and week (P=0.03 for both), whereas this was not found for both rumination time variables (P>0.05). In conclusion, cows grazing during both day and night had a lower methane emission compared to cows fed with spring growth grass-clover silage indoors, which coincided with a longer eating time and shorter rumination time when grazing. Cows grazing during the day and fed with grass-clover silage indoors during the night had methane emission intermediate to the other treatments, while having highest total DMI and ECM yield.

Comparison of two approaches for quantifying methane emissions at housing or individual animal level

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Enteric fermentation in ruminants contributes substantially to of agricultural methane (CH_4) emissions, underscoring the urgent need for accurate quantification of mitigation strategies and to improve greenhouse gas inventory reporting. Increasing demand for precise CH_4 measurements has led to the development of several techniques at individual animal level (e.g. respiration chambers, tracer gas bolus methods, GreenFeed) and at housing level (e.g. tracer-ratio method). This study assessed the accuracy of two approaches for quantifying CH4 emissions from dairy cows. In the first approach using the tracer-ratio method (TR), CH_4 emissions are measured at housing level, which includes animal-based and housing-based emission sources. The tracer gas sulphur hexafluoride was continuously released through a dosing system with critical capillaries positioned adjacent to the aisles. Composite air samples were collected via a PTFE tubing system with critical capillaries. Sulphur hexafluoride concentrations were analysed by gas chromatography and CH_4 concentrations by cavity ring-down spectroscopy. The second approach, the GreenFeed system (C-Lock Inc., Rapid City, SD, USA) quantifies individual enteric CH_4 emissions. Cows were attracted to the GreenFeed units using bait feed. The study involved 20 lactating dairy cows (Brown Swiss and Holstein; average milk yield 33.8 kg/day; body weight 698 kg) kept in a naturally ventilated housing with cubicles and solid floors. The experiment was conducted in early summer 2023 and comprised six sampling weeks (6–7 days each), separated by 3-day intervals, for a total duration of 29 complete days. Cows were fed a partial mixed ration supplemented with concentrate according to milk yield and stage of lactation. All collected raw data from both approaches were consolidated into CH_4 emissions hourly averages (g/h per cow). Original hourly emissions were converted to 4-h rolling means to smooth short-term events and highlight longer-term trends. Data were analysed using R software. The differences in frequency of GreenFeed visits between cows and days of the experiment were assessed using a Kruskal–Wallis test. The differences between both methods were analysed using a T-test and Pearson correlations. Cows visited the GreenFeed unit with a mean frequency of 3.9 ± 1.2 visits/day per cow. Differences between cows' visits were observed ($p < 0.001$). No differences ($p > 0.05$) in visit frequency were found across the days of the experiment. The CH4 emissions per cow were higher when using the TR method (555 ± 114 g CH_4/day) compared with the GreenFeed system (441 ± 50 g CH_4/day, $p < 0.001$). A moderate positive correlation ($r = 0.35$, $p < 0.001$) was observed between the approaches. The two different approaches used to measure the CH4 emissions from a naturally ventilated dairy housing system with the TR method at the housing level and the GreenFeed system at individual level provided consistent realistic values of CH_4 emissions under practical conditions. The higher values obtained using the TR approach may reflect CH4 sources not fully captured by GreenFeed, including hindgut fermentation as well as non-enteric sources such as manure-derived emissions and environmental CH4 . In contrast, GreenFeed enables the assessment of variability between individual animals.

Diurnal profiles of methane emission in response to feed intake patterns of cattle: Implications for GreenFeed measurement sampling schemes

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Gas sampling frequency and timing relative to diurnal CH4 emission and feed intake patterns are critical (Hammond et al., 2016), particularly in experiments using voluntary GreenFeed visits (De Mol et al., 2024). Respiration chamber data analysis showed that accurate quantification of CH4 production (g/d) required sampling at ≤2-h intervals for twice-daily restricted feeding (Van Lingen et al., 2023), whereas sampling frequency and timing did not affect estimates for twice-daily ad libitum feeding. For grazing cattle in particular, feed intake patterns may not be recorded, which makes it difficult to adjust sampling frequency and timing. However, dynamic model simulations could help evaluate diurnal CH4 emissions patterns in response to observed or simulated feed intake rates. The aim was to assess the accuracy of various sampling schemes for estimating CH4 production of dairy cattle that was dynamically simulated for different feed intake patterns. We developed a dynamic model that included differential equations for nutrient degradation, volatile fatty acid production, and methanogenesis in the rumen, based on Van Lingen et al. (2019). Model inputs included two simulated within-day feed intake patterns applicable to grazing cattle and twelve patterns from published studies to simulate diurnal CH4 emission profiles. These studies included 2 to 4 feed deliveries per day. For each simulated diurnal profile, sampling schemes ranging from twice daily to twice per hour were applied to evaluate the accuracy of CH4 production estimates. Simulated diurnal CH4 emission peaks followed the 2 to 4 feed deliveries per day with a delay of approximately 1.0 to 1.5 h, relatively closely aligning with previous work (Van Lingen et al., 2019). Sampling from these simulated diurnal CH4 emissions using intervals ≤ 2h resulted in CH4 productions that did not differ from the areas under the curve across the feed intake patterns. However, samplings that used intervals ≥ 3h or applied only 2 or 3 unequally spaced samplings per day resulted in CH4 productions that differed from the simulated areas under the curve. Preliminary simulations suggested 2-hourly sampling provides optimal CH4 production accuracy across within-day feed intake patterns applicable to grazing cattle and published patterns with 2 to 4 feed deliveries per day. References De Mol, R., Bannink, A., Dijkstra, J., Walker, N., Van Gastelen, S., 2024. The effect of feeding and visiting behavior on methane and hydrogen emissions of dairy cattle measured with the GreenFeed system under different dietary conditions. J. Dairy Sci. 107, 7769-7785. doi: 10.3168/jds.2024-24673 Hammond, K.J., Crompton, L.A., Bannink, A., Dijkstra, J., Yáñez-Ruiz, D.R., O'Kiely, P., Kebreab, E., Eugene, M.A., Yu, Z., Shingfield, K.J., Schwarm, A., Hristov, A.N., Reynolds, C.K, 2016. Review of current in vivo measurement techniques for quantifying enteric methane emission from ruminants. Anim. Feed Sci. Technol. 219, 13-30. doi: 10.1016/j.anifeedsci.2016.05.018 Van Lingen, H.J., J.G. Fadel, L.E. Moraes, A. Bannink, J. Dijkstra. 2019. Bayesian mechanistic modeling of thermodynamically controlled volatile fatty acid, hydrogen and methane production in the bovine rumen. J. Theor. Biol. 480, 150-165. doi: 10.1016/j.jtbi.2019.08.008 Van Lingen, H.J., J.G. Fadel, E. Kebreab, A. Bannink, J. Dijkstra, S. van Gastelen. 2023. Smoothing spline assessment of accuracy of enteric hydrogen and methane production measurements from dairy cattle using various sampling schemes. J. Dairy Sci. 106, 6834-3848. doi: 10.3168/jds.2022-23207

Projecting threshold of annual non-fossil methane emissions at which the atmospheric methane burden equals methane removal

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Recently, we developed a novel method to calculate the balance of cumulative non-fossil methane emissions (BME) over consecutive or non-consecutive livestock production periods. This method enables assessment of whether a (sub)sector or (sub)population (e.g. a dairy region) achieves methane burden or methane removal, by comparing periods and considering methane's atmospheric behaviour in real time. However, among available climate metrics, there is currently no method to project the threshold of current annual methane emissions (AME) required for a sector or population to achieve zero BME at either the population or product level in the current year. As a result, we lack information on how many emissions a production can release without creating additional burden over production periods. Here, we derive and demonstrate equations to project the AME threshold. Below the calculated value, methane is removed, resulting in atmospheric cooling; above it, the methane burden increases, causing atmospheric warming relative to the current temperature. First, we set equations for cumulative methane emissions (CME) in years t and t – 1, taking into account AME and the atmospheric removal of methane over twelve-year periods. The probability that historical methane remains in the atmosphere in real time was calculated taking into account the methane lifetime and the difference in years between the past and the real-time year. Second, for burden equalling removal, we assumed that BMEt, the difference between CME in year t and, in this case, t – 1, equals zero. Third, in the equation BMEt = CMEt – CMEt–1, this means that CMEt equals CMEt–1. Fourth, this assumption enables the use of CMEt–1 instead of CMEt in projecting AMEt. Finally, we derive AMEt threshold from the CMEt equation, where we replace CMEt with CMEt–1. This novel methodology determines the maximum annual methane emissions that may be achieved so that, in the current year, the methane burden does not exceed methane removal, relative to past emission trends and accounting for methane's atmospheric behaviour. Thresholds for future years can be calculated by shifting the periods and considering possible variability in AME. In practice, the developed method can be applied at the population or product level. Furthermore, as with BME, recalculations to carbon dioxide equivalents or carbon dioxide warming equivalents are unnecessary, making the results easier to interpret for non-climatologists. The method aligns with the sustainable development goals and enables the development of tools that animal scientists, regional development institutions, advisory services, breeding and milk recording organisations, or farmers can use to assess the efficiency of methane mitigation measures or short- and long-term production efficiencies at any global livestock population or product level.

Variation in N and P excretion in manure from dairy farms

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In Denmark norm values for N and P excretions ex-storage from livestock are based on mean values obtained from around 70% of all Danish dairy cows. The norm values are used in the field nutrient management plans, that farmers must submit to the authorities. The excretion ex-storage is a mass balance based on mean values for feed intake and composition as well as for milk yield and composition from these farms. Furthermore, constants from literature are used for N and P retention in the body and fetuses, N and P from bedding and N emission from barns and storages. The aim of the study was to examine the variation between farms in the excretion of N and P ex-storage, and to examine if the variation can be explained by individual farm data applied in correction equations provided by authorities. The study was based on data from 1,100 farms with Holstein and crossbred dairy breeds. Data were cured to delete data outside biological relevance. The farms were divided into five percentile groups of equal size based on the level of excretion of N and P ex-animal, respectively. Within each percentile group the mean ex-animal value was calculated as well as mean dry matter intake (DMI), milk yield, and crude protein and P concentration in the feed. For each of the five groups three different emission factors were applied. Within each group it was assumed that 60% had emission compatible with the standard emission for barns with solid floor and automatic scraper at 10.4% of urinary N, and 20% had emission which was either 1.1% higher or lower. Standard amounts of N and P from bedding were added. The emission from outdoor slurry storage was estimated at a constant 3,4% of urine-N ex-barn. The group with lowest N ex-animal excreted on average 140 kg N/cow/y, whereas for the highest group the excretion was 181 kg. Compared to the mean value this was 14% lower and 12% higher, respectively. DMI was 7% lower and 7% higher, respectively, for these two groups, whereas yield of energy corrected milk (ECM) was 5% lower and 5% higher, respectively. Crude protein in DM was 4% lower and 3% higher than the mean. Hence, there was a high variation in N excretion, and DMI was the major driver of N excretion. The group with lowest P ex-animal excreted on average 20.6 kg P/cow/y, whereas for the highest group the excretion was 31.6 kg. Compared to the mean value this was 21% lower and 21% higher, respectively. DMI was 5% lower and 4% higher, respectively, whereas yield of ECM was 5% lower and 3% higher, respectively. The content of P in DM was 11% lower and 11% higher, respectively. Hence, there was a larger variation in P excretion between farms compared to N excretion, and dietary P content was the major driver. The excretion of N ex-storage was estimated at 153 kg/cow/y with a CV of 8.5%, when combining variation in N ex-animal with variation in losses of N from barns. Excretion of P ex-storage was estimated at 26.2 kg/cow/y with a CV of 14.4%. In nutrient planning, farmers are allowed to correct the standard excretions by use of their own data for DMI, crude protein and P in feed, milk yield and protein content in milk in correction equations provided by authorities. When such corrections were used for the highest and lowest groups, the corrected values were at the same level as actually registered for these groups, both for N and P. In conclusion, there is a large variation in N and P excretion between dairy farms, but by using correction equations based on valid data from the individual farm, it seems possible to account for most of the variation.

Feeding strategies to reduce dietary crude protein levels and ammonia emissions on dairy farms

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Ammonia emissions pose a significant environmental and societal challenge in The Netherlands, particularly in the dairy sector, which faces increasing pressure to reduce its contribution. At the same time, national policy is shifting toward results-based policy, emphasizing on-farm environmental targets rather than uniform regulations. Within this context, reducing dietary crude protein (CP) is suggested as a practical and measurable strategy to lower on-farm ammonia emissions. To explore this strategy, the 'Koe & Eiwit' (Cow and Protein) project was conducted from 2022 to 2025. The project involved 150 dairy farms and aimed to reduce CP levels in the diet to 155 g/kg dry matter (DM). On-farm strategies and challenges for lowering CP were examined using data calculated with the Dutch dairy model ANCA (Annual Nutrient Cycling Assessment), covering 2020–2025. ANCA calculates farm-level environmental performance, including CP levels, ammonia emissions and greenhouse gas emissions. Yearly changes in CP were analyzed using a linear mixed-effects model, incorporating annual variation in feed composition, while controlling for farm characteristics and interactions between project period, soil type, and production intensity. Preliminary results indicate an average reduction of 8 g/kg DM in CP from 2020 to 2025, corresponding to an 8% decrease in ammonia emissions. Key drivers included lower CP levels in grass silage (due to fertilization adjustments and mowing timing), increased maize feeding, and reduced CP levels in concentrates. Reductions were achievable across farms, although peat soils and grass-based systems posed greater challenges. No significant effects were observed on milk production, feed costs, or other environmental indicators. The results highlight feeding strategies for lowering CP and identify farm characteristics that may require tailored targets and support. They provide guidance for policymakers and farmers to achieve environmental goals while maintaining production and animal health and safeguarding vulnerable natural habitats.

Long-term effects of reduced dietary protein supply in lactating dairy cows

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Current feeding practices in dairy systems tend to overfeed protein in relation to the predicted milk production response. However, few studies have addressed the long-term effects of providing protein at levels currently considered below requirements. This experiment aimed to evaluate the effects of diets formulated around 5% below current protein recommendations provided by the Nordic Feed Evaluation System (NorFor) with varying levels of metabolizable protein (MP) and rumen protein balance (RPB) on lactational performance over the long term. A total of 72 mid-lactation Danish Holstein cows, 36 multiparous and 36 primiparous [averaging (mean± SD) 119 ± 57.6 and 138 ± 65.2 days-in-milk and 41.2 ± 5.4 kg/d and 32.0± 3.1 kg/d milk yield, respectively], were included in a 15-week complete randomized block design experiment preceded by a 2-week covariate period. During the covariate period, all cows ate the ration fulfilling protein recommendations according to NorFor (MP: 15.5 g/MJ, RPB: 12 g/kg DM). A total of 9 dietary treatments in a 3 × 3 factorial arrangement with 3 levels of MP (15.5, 14.5, and 13.5 g/MJ) and 3 levels of RPB (12, 4, and -4 g/kg DM) were formulated by varying maize gluten, protected soybean meal (SoyPass), sunflower meal, lupin pulses, and urea. The diets were fed as TMR and offered ad libitum. Intake was recorded daily using automated feed bins (RIC; Insentec BV) with each cow randomly assigned to one bin throughout the study. Cows were milked twice daily and daily milk yield (MY) was recorded. Milk samples were collected weekly from 2 milkings. Dry matter intake (DMI), energy-corrected milk yield (ECM), and milk protein yield were averaged by week and analysed using the nlme function in R with a model including covariate week and fixed effects of parity, MP level, RPB level, experimental week, and interactions among MP, RPB and week. Experimental week within cow was used as a repeated term with an autoregressive residual correlation structure. The interaction between MP, RPB and experimental week were not significant for any of the tested variables. Overall, DMI did not differ among MP or RPB levels and averaged 21.7 ± 0.26 kg/d across parity groups. However, there was an interaction between RPB and experimental week (P = 0.03), it being lower for the lowest RPB level in the first experimental weeks, recovering to a similar level across the RPB levels towards the end of the experiment. When taking into consideration the duration of the trial, a lower RPB may have limited microbial protein synthesis and ruminal fermentation during the first weeks of the experiment, and therefore limited DMI, whereas in later experimental weeks the rumen may have had time to adapt to the lower levels of RPB and recovered DMI to a similar level across the RPB levels. Energy-corrected milk yield was affected by MP overall (P = 0.01), but the response was neither linear nor quadratic. Adjusted means suggested lower ECM (33.1 ± 0.79 kg/d) at the lowest MP level (13.5 g/MJ), whereas the intermediate and highest levels (14.5 and 15.5 g/MJ) did not differ (35.0 vs 34.7 ± 0.79 kg/d; overall MP effect, P = 0.01). Rumen protein balance did not affect ECM. Milk protein yield decreased in a linear manner (P = 0.01) with both decreasing MP and RPB from 1.20 to 1.13 kg/d for MP and from 1.21 to 1.14 kg/d for the RPB levels, respectively. In conclusion, these preliminary findings indicate that a moderate reduction of dietary protein supply is possible in dairy cows' diets, without compromising overall production and feed intake. Further, the distinguishing between metabolizable protein and rumen protein balance still remain important for sustaining the overall production of the animals.

Quantitative meta-analysis on the effects of manipulating rumen degradable protein balance by changing protein degradability on fat and protein corrected milk in Holstein dairy cows

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Inefficiency of nitrogen (N) utilization in dairy cattle production results primarily from N losses in urine and feces. These losses are partly attributed to an imbalance between rumen-available N and fermentable energy, which limits microbial capture of ammonia in the rumen. RDPB, calculated according to the DVE/OEB system (Van Duinkerken et al., 2011), represents the balance between microbial protein synthesis potential from rumen degradable protein and that supported by fermentable energy. A positive rumen degradable protein balance (RDPB) indicates potential N loss from the rumen, while a negative RDPB suggests microbial protein synthesis may be impaired due to an N shortage. We performed a meta-analysis to quantify the effect of changing RDPB, through changes in protein degradability, on fat and protein corrected milk (FPCM). A literature search covered PubMed, Google Scholar, ScienceDirect, Web of Science, and CABI. Inclusion required clear descriptions of animal characteristics (milk yield and composition, dry matter intake, body weight) and diet composition. To isolate the effect of RDPB, we retained studies where intestinal digestible protein (DVE) was kept relatively constant while RDPB varied due to changes in rumen degradable protein supply from protein-rich feedstuffs. The final dataset comprised 206 observations from 56 studies. Milk yield was standardized to FPCM using: FPCM (kg/d) = milk (kg/d) * (0.337 + 0.116 * fat% + 0.060 * protein%). Mean body weight, DMI, and FPCM were 638 kg, 23.2 kg/d, and 33.3 kg/d. Statistical analysis used exponential mixed models in SAS (GLIMMIX and NLMIX), with study as a random effect: FPCM = a + b * (1 – exp(-c * RDPB)). Predictors were RDPB (g/kg DM) and RDPB intake (RDPBI). Models were evaluated using mean square prediction error (MSPE), RMSPE, and concordance correlation coefficients (CCC). Both the RDPB and RDPBI models performed well (R2 = 0.88; CCC = 0.93). The FPCM response to RDPB was curvilinear (a = 33.01, b = 1.376, c = 0.0350; $P < 0.05$). Model fit was good (RMSPE = 6.82; AIC = 1164.9). Increasing RDPB from -20 to 0 g/kg DM increased FPCM by 1.4 kg/d, nearly double the 0.69 kg/d increase from 0 to +20 g/kg DM. The response flattened at approximately 20 to 25 g RDPB/kg DM. In conclusion, RDPB levels significantly influence FPCM via a nonlinear relationship. Maintaining a non-negative RDPB is important for rumen function and production, while increasing RDPB far beyond zero offers diminishing returns for milk output and may increase environmental N losses. Reference: van Duinkerken, G., Bannink, A., Blok, M. C., Cone, J. W., Dijkstra, J., Tamminga, S., & van Vuuren, A. M. (2011). Assessment of the DVE/OEB system for protein evaluation in ruminants. In 36th Animal Nutrition Research Forum-A platform to present Animal Nutrition Research in Belgium and the Netherlands, Heverlee, Belgium, 19 april, 2011.

Impact of diet optimisation on diet carbon footprint intensity and productive performance of lactating dairy cows

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Milk carbon footprint intensity (MCFI) is influenced by emissions associated with dietary ingredients. We hypothesized that diet optimization can improve MCFI by reducing diet carbon footprint intensity (DCFI, kg CO2e/kg fat and protein corrected milk (FPCM)) where DCFI is based on FPCM and ingredient intake and emission factors. Ingredient and cow data were used by 2 models to optimize diets relative to a control diet formulated to reflect commercial conditions (CON): Trouw Nutrition Response research model (OT1) and Cornell Net Carbohydrate and Protein System v6.5.5 (CNCPS, OT2). A prior Brazil study evaluated commercially available ingredients not currently used by enrolled commercial farms (dried distillers grain with solubles, urea, rumen protected methionine, rumen protected lysine); these were optional in the current study optimisations, partly replacing other ingredients. The study was a randomized complete block design in Brazil (28 d adaptation and covariate, 56 d experimental). Forty-five Holstein cows (20% primiparous, 80% multiparous; 36.0 ± 7.5 kg/d milk yield (MY); 133 ± 57 days in milk (DIM)) were enrolled, paired by parity, MY, DIM and body weight, and randomly assigned to treatment. Data analysis used a mixed model with treatment, week and their interaction as fixed effects and block as a random effect. Least squares means were compared using Tukey's test. Significance and trends were declared at $P \leq 0.05$ and $0.05 < P \leq 0.10$, respectively. Based on covariate data (including observed DMI of 27.4 kg/d) and subsequent diet optimisations, predicted MY was 35.7 and 54.2 kg/d, whereas predicted DCFI was 1.03 and 0.64 for OT1 and OT2, respectively. Dry matter intake was lower in OT1 vs. CON and OT2 (25.6; 26.3; 26.6 kg/d) but did not differ in CON vs. OT2. Milk yield was lower in OT1 vs. CON and OT2 (35.3; 36.2; 36.4 kg/d) and FPCM responded similarly (31.5; 33.5; 33.3 kg/d). Milk protein content was higher in OT2 vs. CON and OT1 (3.63%, 3.51%; 3.45%) and CON vs. OT1 tended to differ. Milk fat content was higher in CON vs. OT1 and OT2 (3.36%; 3.10%; 3.14%). The DCFI differed in OT1 (-11.3%) and OT2 (-21.8%) vs. CON (1.18; 1.04; 1.33 kg CO2e/kg FPCM) and differed in OT1 vs. OT2. Milk urea nitrogen was lower in OT1 (-11.2%) and OT2 (-25.7%) vs. CON. Nitrogen utilization efficiency (NUE) was higher in OT1 (+4.5%) and OT2 (+16.9%) vs. CON, supported by different dietary crude protein levels (18.0%; 16.9%; 16.2% of dry matter for CON, OT1 and OT2). Feed efficiency (FPCM–based) was lower in OT1 vs. CON and OT2 (1.23; 1.28; 1.26) and tended to differ in CON vs. OT2. Relative to model predictions, observed MY was 1% and 33% lower, while DCFI was 15% and 63% higher for OT1 and OT2, respectively, the latter partly explained by 8% higher DCFI for CON in experimental vs. covariate phases. Differences in predicted vs. observed outcomes merit further research. Diet optimization had variable effects on productivity. For both OT1 and OT2, we confirmed the hypothesis that diet optimization can improve DCFI, while NUE also improved. Ongoing research is evaluating economics and enteric methane and manure emissions to confirm effects on MCFI.

Grazing, feeding, manure and air management for dealing with environmental impacts of intensive dairy farming

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Intensive dairy farming is increasingly challenged to reduce environmental emissions while maintaining productivity and economic viability. This contribution focuses on practical farm-level strategies and emerging technologies that can support this transition. A first pathway concerns grazing and feeding management. In the Netherlands, sector agreements have aimed to stimulate grazing and reduce crude protein content in dairy cow rations. However, implementation of more grazing remains challenging, particularly for the increasing number of herds using automatic milking systems. Lowering dietary crude protein has proven effective in reducing nitrogen losses and ammonia emissions in a field project set-up, but further progress requires improved farm-specific ration formulation and monitoring; expansion of this activity of lowering crude protein to the whole dairy farm sector is in progress. A second pathway is the transition toward target-oriented environmental management, in which farmers can combine practices and technologies to meet farm-specific emission goals. This approach requires reliable on-farm sensors for monitoring emission outflows; the current state of development of such tools will be discussed. A third pathway involves innovations in housing, manure and air management. Systems such as the cow toilet and Lely Sphere illustrate the movement from experimental concepts to practical application. In addition, our work addresses the combined removal of ammonia and methane from barn air. We currently evaluate new options such as the Methane Eradication Photochemical System (MEPS), developed at Copenhagen University, which has shown reductions of more than 90% for NH3 and about 50% for CH4 under experimental conditions. In parallel, cooperation with the University of Washington explores methanotroph bacteria for methane digestion at low concentrations (90% CH4 reduction at 500 ppm; CH4 concentrations in dairy barns vary from 5 to 100 ppm). The potential valorization of the N captured from manure and air in housing facilities, to be used as fertilizer, and the bacteria digested methane to protein, envisioned to become useful as animal feed, are essential for the economic result of these new technologies. Wageningen University & Research supports and plans implementation through demonstration and system redesign at two locations: an emission-free, labour-efficient dairy housing facility at Dairy Campus, and a lower-input dairy system with precision grazing, precision feeding, and freewalk housing with compost bedding at Agro-Innovation Centre De Marke. These examples show that reducing environmental impacts in intensive dairy farming will depend on integrated, farm-specific combinations of management, monitoring, gas emission capture and nutrient valorization.

Farm level analysis of on-farm monitoring of methane from dairy cows and relationship to farm characteristics and farm level climate accounts

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The aim of the present study was to analyze methane measurements obtained from on-farm monitoring of dairy cows in Denmark using the Sniffer-method in AMS systems during milking to identify farm characteristics associated with variation in average methane emissions between farms. Furthermore, on-farm methane measurements were compared with methane emissions per cow as calculated in the Arla farm-level climate accounting tool. The analysis was based on data from 24 dairy farms, each contributing between 1 and 4 years of observations from the period 2021–2024. The dataset included cows of the Holstein, Danish Red, and Jersey breeds. For the analysis, methane data were aggregated as annual farm-level averages, and the following variables were included: measured CH_4 concentration (ppm) in expired breath, calculated kg CH_4/cow/day, and calculated kg CH_4/kg ECM. These annual methane averages were further stratified into two groups: first-parity cows, and cows in second or higher parity. Each farm was visited once by a technician, who conducted interviews to collect information on farm characteristics. Key variables hypothesised to influence methane emissions included: The proportion of roughage in the ration (% of DM), the composition of roughage (proportion of grass silage and grazed grass), the strategy for concentrate allocation (e.g., individually allocated vs. group-based), and the amount of concentrate provided per cow. Using routinely recorded farm performance data, several key performance indicators (KPIs) were calculated, including milk yield per cow per year, culling rate, average age of cows in the herd, cow mortality, incidence of hoof and limb disorders, metabolic disorders and mastitis, as well as live weight. The analysis examined whether variation in these KPIs could explain variation in methane emissions among farms. On-farm methane measurements were compared with methane emissions per cow estimated in the climate accounting tool from Arla. The hypothesis was that the ranking of farms in terms of methane emission per kg of milk would be similar whether methane was directly measured on-farm or estimated through the climate account. Measured enteric methane emissions per cow per day were increased with increased milk yield per cow per day. Whereas g CH4 per kg ECM declined with increased milk yield across all three breeds. This was the case both when presented per breed and across all three breeds.

Carbon farming potential of Mediterranean cow–calf systems under different cultivation intensities

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This study aimed to estimate the net carbon footprint (Net-CFP) of Mediterranean cow-calf systems and to assess how cultivation intensity affects their carbon farming potential. Eighteen beef farms adopting a cow-calf grazing system, with an open productive cycle, were considered. Farms were selected in relation to the cultivation rate (CR) and classified as: high (high-CR; > 15% of cultivated lands) and low (low-CR; <15% of cultivated lands). Data were collected by interviewing farmers, in a cradle to farm gate approach, and according to ISO14040:2006 and 14044:2006 standards. The average of five producing years was chosen as the temporal boundary. Emission intensity was reported as kilogram of CO2 equivalent (CO2e) per kg of live weight (LW) sold from yearling beef, per kg of total LW (TLW) sold from end-career cows, bulls, and yearling beef, and per hectare (ha) of land. Soil carbon sequestration was estimated by considering the above- and below-ground biomass residues, and the organic carbon from manure deposition. Carbon sequestration from Meriagos, forests, and Mediterranean scrubland was also considered. Gross CFP was lower in low-CR farms than in high-CR farms (19.80 vs. 26.75 kg CO_2e/kg LW sold). Enteric methane was the main contributor, accounting for 68.3% and 74.2% of total GHG emissions in high and low-CR farms, respectively. When carbon sequestration was included in the model, Net-CFP was lowest ($P<0.01$) in low-CR farms. In conclusion, lower cultivation intensity reduced the CFP of cow-calf system, generating higher carbon credits that could fully offset the emissions of the fattening phase. These results highlight the carbon farming potential of extensive Mediterranean beef system, where practices that increase soil carbon sequestration can enhance sustainability and support EU climate policies. However, this potential is based on farm level assessments and does not automatically translate into certified carbon credits. Therefore, further work is needed to assess eligibility and practical implementation within formal carbon crediting frameworks. Acknowledgements The authors acknowledge the University of Sassari for the financial support (Mobilità Giovani Ricercatori, annualità 2024-2025; D.R. rep. n. 4422/2025, prot. n. 148562 del 23/12/2025).

Seasonal variation in enteric methane emissions and production efficiency metrics in a higher-output Irish pasture based dairy system including concentrates with differing levels of crude protein.

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Quantifying seasonal variations in enteric methane (CH4) and its relationship with production metrics allows for the development of more accurate, system-specific conversion factors for pasture-based dairy systems. Accurate CH4 conversion factors are essential for correct national greenhouse gas inventories, yet current Irish estimates rely largely on predictive Tier 2 models rather than direct measurement . This approach poorly captures the variation due to season, diet, grazing conditions or cow types, applicable to Irish pasture-based systems. Furthermore, Irish farmers are required to restrict concentrate crude protein (CP) to a maximum CP of 14% during the main grazing season . The effects of concentrate CP concentration, and the associated response in enteric CH4 production in grazing dairy cows, are not established. Therefore, the objectives of the study were to 1) directly measure enteric CH4 across the grazing season and 2) quantify the effect of concentrate CP content on enteric CH4 production in a higher output grazing system. Fifty spring-calving Holstein-Friesian dairy cows were blocked by parity and days in milk (DIM). Within blocks, primiparous cows were balanced by body weight (BW) and pre-experimental milk solids (MS) , while multiparous cows were balanced by their previous 305-day MS and milk yield. Cows were randomly allocated to one of two dietary treatments: a 12% (C12) or 14% (C14) CP concentrate formulated with barley, oats, and beans. The concentrate supplementation (CS) rate was adjusted based on DIM, decreasing from 8 kg/d in early lactation to 3 kg/d in late lactation. The herd grazed a 17.35-ha farmlet as a single group, receiving their allocated concentrates individually during twice-daily milking. Daily pasture allowances ranged from 14 to 17 kg dry matter (DM) per cow, adjusted for stage of lactation and CS levels. The 17-week study (April 28–August 22) was preceded by a 3-week acclimatization period. Enteric CH4 emissions were measured continuously using three GreenFeed units (GEM). Pasture intake was estimated via the n-alkane marker method during weeks 6 (Period 1), 12 (Period 2), and 19 (Period 3). Data was analysed using repeated measures ANOVA (MIXED procedure). A probability of $P \leq 0.05$ was selected as the level of significance, and statistical tendencies were reported when $0.05 < P \leq 0.10$. Neither milk yield nor MS production was affected by concentrate CP concentration. Daily milk yield was 32.6 kg/d and 33.2 kg/d for cows offered C12 or C14 respectively. Pasture dry matter intake (DMI) was unaffected by treatment ($P = 0.722$), though it did vary between period ($P < 0.001$). Mean pasture DMI per cow was 15.1 kg DM, 17.8 kg DM and 17.6 kg DM in period 1, 2 and 3 respectively. Mean enteric CH4 production was lower ($P = 0.05$) for cows offered C12 (323 g/d) than C14 (343 g/d). CH4 expressed per kg of milk, MS, DMI or BW was not affected by treatment. However, both enteric CH4 emissions and intensity metrics increased from period 1 to period 3 ($P < 0.05$). Absolute CH4 production rose from Period 1 to Period 3, a trend mirrored by CH4 yield (CH4 /kg DMI) and CH4 intensity (CH4/kg MS). These results show that enteric CH4 in higher-output grazing systems varies seasonally and highlight the need for season-specific conversion factors.

Environmental impact of pasture turnout strategies in dairy cows grazing timothy–meadow fescue pasture

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Grazing animals affect pastoral systems by removing nutrients during grazing and redistributing them through excreta. In grazing systems, urinary N deposition can exceed plant N uptake, leading to nitrate leaching, ammonia volatilization, and nitrous oxide emissions that contribute to atmospheric pollution. Moreover, data on enteric methane (CH4) emissions from grazing systems are often inconsistent and vary with experimental conditions. This study investigated the impact of early-season part-time grazing on urinary N and urea excretion and CH4 emissions in dairy cows in northern Sweden. The experiment followed a randomized block design with 40 lactating Nordic Red cows. During a 14-day covariate period, cows were housed indoors and fed a TMR ad libitum containing grass silage and concentrate (60:40 forage-to-concentrate ratio on a DM basis). During the subsequent 28-day treatment period, cows within blocks were randomly assigned to 1 of 4 treatments: indoor feeding only (CON), 12 h grazing with ad libitum indoor feeding (G1), 12 h grazing with restricted indoor feeding (80% of DM intake during the covariate period; G2), and 12 h grazing with ad libitum indoor feeding and a 50:50 forage-to-concentrate ratio in the TMR (G3). The pasture was sown with 10% white clover, 40% timothy, 40% meadow fescue, and 10% Kentucky bluegrass, and herbage allowance exceeded 20 kg DM/cow per day. Spot urine samples were collected once daily for 3 consecutive days at the end of each period and pooled within cow and period. Urinary creatinine was used to adjust urea and N concentrations for urine dilution. These data were used to estimate excretion of urinary N (UNe) and urinary urea (UUNe). Enteric CH4 and CO2 emissions were measured using two indoor GreenFeed (GF) units (C-Lock Inc., Rapid City, SD), with no GF available on pasture, and data from the final 14 days of each period were used for analysis. Records with <20 GF visits per cow during the measurement period were excluded. Data were analyzed using analysis of covariance (ANCOVA) in SAS, including treatment and block as fixed effects and baseline value as a covariate. When treatment effects were significant, the following contrasts were specified: CON vs. G1 and G2; CON vs. G3; and G1 and G2 vs. G3. Because cows in G2 did not reach the intended restriction level, they were considered ad libitum and therefore analyzed together with G1. Treatment did not affect urinary urea or N concentration, UNe or UUNe ($P \geq 0.14$). Mean UNe values were 261 ± 19, 212 ± 16, 246 ± 17, and 256 ± 16 g N/d for CON, G1, G2, and G3, respectively. Daily CH4 emissions were lower ($P < 0.01$) in G1 and G2 than in CON (368 ± 11 and 375 ± 11 vs. 415 ± 12 g/d, respectively), whereas daily CO2 emissions did not differ among treatments. Methane intensity did not differ among treatments and averaged 11.4 ± 0.4 g/kg ECM. Several limitations should be considered. Grazing cows had access to GF only during daytime indoor periods, with no GF available on pasture, with no measurements during the 12 h night grazing period, limiting the ability to capture diurnal variation in CH4 emissions related to feeding patterns. In addition, urinary volume was estimated from pooled spot urine samples, which may have introduced uncertainty in the estimates. Within the conditions of this study, early-season part-time grazing did not affect urinary urea or N excretion. Although daily CH4 emissions were lower in grazing cows receiving the same daytime diet as indoor-fed cows, CH4 intensity was similar between indoor-fed cows and part-time grazing cows.

Forage type and lipid-rich concentrates as practical levers to mitigate enteric methane in fattened lambs: links to rumen fermentation and methanogenic microbiota

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Enteric methane (CH_4) from ruminants remains a key contributor to the environmental footprint of livestock systems. Practical mitigation strategies that can be implemented at the farm level, through feeding and forage management, are therefore urgently needed. This study evaluated how forage type (grass vs. red clover) and a lipid-rich concentrate influence rumen fermentation, microbial ecology, and CH_4 production in fattened lambs, providing evidence for an applicable dietary approach to reduce emission pressure. The experiment was conducted over two consecutive years (2022 and 2023) and involved a total of 48 ram lambs (24 lambs per year), fattened to a body weight of approximately 35 kg (± 3 kg). Animals were assigned to four dietary treatments based on concentrate offered at 3% of body weight and forage provided ad libitum: (1) control: standard cereal-based concentrate (>50%) with rapeseed + soybean meal (>40%) plus grass; (2) "OL" concentrate containing 35% oily components (15% rapeseed cake, 15% DDGS, 5% linseed) plus grass; (3) standard concentrate plus red clover; (4) OL concentrate plus red clover. Rumen fluid was collected post mortem from all 48 lambs to determine pH, volatile fatty acid (VFA) profiles, ammonia concentration, and the abundance of bacteria, protozoa, and methanogens. Methane production (g CH_4/day) was measured at the final stage of fattening using respiration chambers equipped with infrared gas detection (Servomex 4000 Series, UK). Measurements were performed on a subset of animals representing 4 groups × 2 lambs × 2 experimental repetitions. In total, 16 lambs were evaluated, with 8 animals measured in each year. Dietary inclusion of oily components modified the rumen microbial profile, as reflected by a lower protozoal count (10.3 × 10^5/mL vs 18.2 × 10^5/mL in the control) and a shift in fermentation toward greater propionate production (16.8 vs 13.0 mM/L). These changes were accompanied by a reduction in methane emission. The greatest mitigation effect was observed in lambs receiving the red clover-based diet combined with the OL concentrate, where methane emission was reduced by 20.5%, from 19.6 g CH_4/day in the control group to 15.6 g CH_4/day. Our findings indicate that combining legume forage (red clover) with a lipid-enriched concentrate can deliver meaningful (by ~20%) CH_4 mitigation in intensive lamb fattening, likely through coordinated effects on rumen fermentation pathways and microbial communities involved in methanogenesis.

Evaluation of dibromomethane as an anti-methanogenic feed additive for dairy cows in a dose-response study

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Bromoform, a trihalomethane, has been identified in numerous studies as an effective methane-mitigating feed additive, with most studies focusing on bromoform derived from the red seaweed Asparagopsis spp. However, large-scale cultivation or harvesting and subsequent post-harvest processing of Asparagopsis spp. presents practical challenges. Moreover, concerns regarding animal and consumer health have been raised, as bromoform is classified as a possible human carcinogen. Studies using Asparagopsis spp. have also reported elevated bromine concentrations in milk. Dibromomethane (DBM), a dihalomethane, has likewise demonstrated anti-methanogenic effects in vitro. In theory, the use of DBM as a methane-mitigating feed additive may result in lower bromine transfer to milk compared with the trihalomethane. Therefore, this study investigated the effects of increasing doses of DBM in the feed ration of dairy cows on enteric gas emissions, dry matter intake (DMI), and energy-corrected milk (ECM) yield. It was hypothesized that increasing levels of DBM would result in greater inhibition of ruminal methanogenesis without affecting DMI or milk yield. The experiment was conducted as a 4 × 4 Latin square using four multi-cannulated Danish Holstein dairy cows. According to a balanced Williams design, the cows received either a control ration without additive (CONTROL) or the same ration supplemented with 7 (LOW), 14 (MEDIUM), or 21 mg DBM/kg DM (HIGH). Each period lasted 21 d and consisted of 14 d of adaptation, followed by 3 d of digesta sampling and 4 d of gas exchange measurements using respiration chambers. The rumen was visually inspected for signs of damage, such as papillae atrophy or ulcers, prior to the experiment and on d 17 of each period by rumen evacuation. Milk yield and feed intake were recorded daily, DMI was determined from d 14 to 21, and milk solids were analysed from d 18 to 21. All parameters were statistically analysed using a mixed procedure of R. The addition of DBM had no effect on DMI (kg/d; linear effect: $P = 0.24$), whereas ECM yield (kg/d) tended to decrease linearly with increasing DBM dose ($P = 0.09$). Daily methane emission (g CH_4/d), yield (g CH_4/kg DMI), and intensity (g CH_4/kg ECM) decreased with increasing DBM dose ($P < 0.01$, $P = 0.02$, and $P = 0.03$, respectively). In contrast, daily hydrogen emission (g H_2/d), yield (g H_2/kg DMI), and intensity (g H_2/kg ECM) increased with increasing DBM dose ($P < 0.01$ for all parameters). No indications of rumen damages were observed (visual inspections) during rumen evacuations. Methane yield and intensity were reduced by 16% and 15%, respectively, from CONTROL to HIGH, whereas similar doses of bromoform in seaweed studies have resulted in 25–30% reductions in dairy cows. Hence, the efficacy of DBM appears to be lower than that of bromoform at the same halomethane inclusion level (mg/kg DM). In the rumen, both bromoform and DBM are rapidly metabolized. However, because bromoform is converted to DBM as an intermediate, methanogenesis is inhibited for a longer duration following bromoform supplementation than with DBM alone, which may explain its greater methane-mitigating potential. In conclusion, increasing DBM dose resulted in a linear inhibition of rumen methanogenesis; however, a tendency for a linear decrease in ECM yield was also observed, while DMI was unaffected.

Grape stem inclusion in the diet of fattening young bulls reduced methane emissions

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To promote circularity, grape (Vitis vinifera) stems, a fibrous agro industrial by product, can be included in fattening cattle diets at low doses (up to 6%) without deleterious effects on performance. The polyphenols present in grape have antioxidant and anti-inflammatory effects, and besides they could reduce the methane emissions modifying rumen fermentation. Young bulls (162 days old; 173 kg BW, n=24) were fed iso-energetic (13.2 MJ/kg DM) and iso-proteic (11.2% CP) concentrates plus barley straw ad libitum for 17 weeks. Concentrates contained different proportions of grape stem and barley straw in inverse ratios: 0% stem (6% straw), 3% stem (3% straw) and 6% stem (0% straw). Young bulls were weighed weekly to monitor body weight (BW) and calculate average daily gain (ADG). Concentrate intake per animal was registered with two ALPRO feeding stations (ALPRO Herd Management 7.0, DeLaval) to estimate dry matter intake (DMI). Methane concentrations were recorded with two wall-mounted Guardian NG NDIR methane monitors (Edinburgh Instruments, Livingston, UK) sampling air continuously from the concentrate feed bins via a fixed inlet tube. Average weekly methane emissions were derived by converting the sniffer-measured CH_4:CO_2 concentration ratio into daily CH_4 output using an estimated daily CO_2 production from animal heat production (based on BW and ADG). Methane emissions were analysed using a mixed-effects model (PROC MIXED, SAS), including grape stem inclusion level, week and their interaction as fixed effects, with the animal as a repeated subject (ARH(1) covariance structure), Kenward–Roger adjustment for degrees of freedom, and Tukey-corrected pairwise comparisons of least squares means. Compared with the control diet (0% grape stem), both grape-stem inclusion levels (3% and 6%) reduced daily methane production by approximately 10% (146, 131 and 131 g/d for 0%, 3% and 6% respectively; $P < 0.05$). When methane emissions were expressed per unit of DMI, only the 6% inclusion level remained lower than the control, whereas the 3% level was intermediate (19.4, 18.0 and 17.5 g/kg DMI, respectively ($P=0.03$). In contrast, expressing methane relative to BW reduced the magnitude of treatment differences and no significant contrasts with the control were detected (0.364, 0.339 and 0.340 g/d kg BW; $P>0.05$), suggesting that part of the reduction in absolute emissions was associated with differences in intake and animal growth rather than a uniform shift in methane yield. This lack of significance is likely due to the limited sample size and the variability in methane measurements. The inclusion of grape stems in high-concentrate diets for fattening young bulls reduced methane emissions. However, the magnitude and statistical significance of the reduction depended on how methane production was expressed. Further studies with larger sample sizes and more precise methane measurements are needed to confirm these effects.

Impact of sainfoin (Onobrychis viciifolia) inclusion in beef fattening diets on methane emissions

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Improving feed self-sufficiency in beef production is essential to reduce economic vulnerability and the environmental footprint associated with imported protein sources such as soybean meal. Sainfoin (Onobrychis viciifolia, SF), a tannin-rich legume adapted to local conditions, may enhance nitrogen (N) utilization while reducing enteric methane (CH_4) production. We hypothesized that increasing dietary sainfoin inclusion would modify CH_4 emissions depending on its NDF content and starch-to-NDF ratio of the diets. Forty Montbéliarde calves (117 days; 188 kg initial BW) were allocated to four diets in a 2 × 2 factorial design over 28 weeks, differing in forage type (barley straw vs. sainfoin hay) and concentrate formulation (control vs. sainfoin concentrate). The sainfoin concentrate was formulated by including 15% pelleted sainfoin, mainly replacing fibrous by-products and part of the cereal–protein fraction of the control concentrate (notably wheat bran, soybean meal, rapeseed meal and barley), while maintaining a similar cereal base based on corn grain. Treatments were: C+straw (control concentrate + straw, 0% SF in the total diet), CSF+straw (sainfoin concentrate + straw, 12% SF in the diet), C+SFhay (control concentrate + sainfoin hay, 20% SF in the diet), and CSF+SFhay (sainfoin concentrate + sainfoin hay, 33% SF in the diet). Individual intake and BW were recorded daily automatically and were used to calculate feed efficiency (growth:feed). Methane concentrations were recorded with a wall-mounted Guardian NG NDIR methane monitor (Edinburgh Instruments, Livingston, UK) sampling air continuously from the concentrate feed bin via a fixed inlet tube. Average weekly methane emissions were derived by converting the sniffer-measured CH_4:CO_2 concentration ratio into daily CH_4 output using an estimated daily CO_2 production from animal heat production (based on BW and ADG). Each treatment was recorded during one month. Methane emissions were estimated using mixed linear models (lmer, R), including animal as random effect and second-order polynomial BW adjustment. Methane production increased as dietary starch:NDF ratio decreased. Absolute CH_4 emissions were highest in CSF+SFhay (80.9 g/d) and lowest in CSF+straw (65.8 g/d). The increase of emissions observed exceeded the expected increase according to starch:NDF ratio in CSF+SFhay and C+straw (1.9 and 2.2 respectively), suggesting a stronger-than-expected NDF effect from sainfoin. On the other hand, CSF+Straw had equal methane emissions than C+Straw despite a higher NDF content. The random effect obtained from the model had a low negative correlation with individual efficiency ($r=-0.31$, $p<0.05$). In conclusion, a high inclusion of sainfoin (33%) increased enteric CH_4 emissions due to the greater dietary NDF associated with higher forage intake, whereas at a medium inclusion level (20%) this increase was partially counteracted by the greater intake of sainfoin-derived tannins. Based on the analysed tannin concentration of sainfoin hay (3.96 g equivalents condensate tanins/kg DM) and measured feed intake, estimated tannin intake increased from 0.0 to 3.2, 4.0 and 10.0 g/d in the 12%, 20% and 33% sainfoin diets, respectively. Therefore, when maximizing circularity is the main objective, the combined inclusion of sainfoin in pellet and hay form is recommended, whereas a moderate inclusion through pellets with straw may be preferable when prioritizing absolute CH_4 mitigation.

Optimizing fattening duration in Hanwoo steers: Impacts on growth performance, enteric methane emissions, feed efficiency, and rumen microbial communities

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Methane (CH4) from ruminant enteric fermentation contributes significantly to anthropogenic greenhouse gases, particularly Hanwoo beef production in Korea, relying on prolonged fattening (>30 months) for marbling, amplifying emissions. This study evaluated the impact of fattening duration on growth performance, greenhouse-gas (GHG) emissions, and rumen microbial communities in Hanwoo steers. Forty-eight Hanwoo steers were assigned to six groups (n=8 per group) by starting age (10 or 13 months) and slaughter age (26, 28, or 30 months): 10/26 , 10/28, 10/30, 13/26, 13/28, and 13/30. Steers were monitored for body weight (BW), dry matter intake (DMI), GHG emissions (CH4 and CO2), and rumen fermentation profiles. Gas measurements were meaured using the GreenFeed system (C-Lock Inc., Rapid City, SD, USA) over three consecutive days at slaughter age, following an 8 spot-sampling protocol to cover a 24-h feeding cycle: 0900, 1500, 2100 h (day 1); 0300, 1200, 1800 h (day 2); and 0000, 0600 h (day 3). At slaughter age, body weight was measured, and rumen fluid was collected using an oral stomach tube connected to a vacuum pump. Collected samples were used to analyze the fermentation and rumen microbiota profiles. Microbial composition was characterized by 16S rRNA amplicon sequencing of the V3-V4 (bacteria) and V5-V6 (archaea) regions. Data were analyzed using a two-way fixed-effects model implemented in SAS (PROC MIXED). The model included two categorical fixed effects: starting age (10 or 13 months) and slaughter age (26, 28, or 30 months), along with their interaction. Statistical significance was declared at $P \leq 0.05$. No significant differences were observed in rumen fermentation parameters across groups ($P > 0.05$). The slaughter age showed a tendency to influence final body weight (PSlaughter age = 0.0532) across groups, with the highest BW recorded in the 10/30 group (835.00 ± 30.00 kg). Additionally, the 13/28 group exhibited greater feed efficiency, with the lowest feed conversion ratio (FCR = 11.16 ± 0.81) and a negative residual feed intake (RFI = -1.91 ± 0.30) compared to other groups. Total CH4 and CO2 emissions revealed significant interactions between starting and slaughter ages ($P < 0.05$). The 13/28 group had the lowest total CH4 emissions (70.60 ± 8.07 kg CH4 /head) and CO2 (3942.70 ± 443.55 kg CO2/head) compared to other groups ($P< 0.05$). Additionally, the 13/28 group exhibited the lowest daily CH4 production (g/d) and CH4 yield (g/kg DMI) than other groups ($P < 0.05$). Microbial analysis identified Bacteroidota and Bacillota as dominant bacterial phyla and Methanobacteriota as the primary archaeal phylum. Ruminococcus bromii ($r = 0.80$, $P = 0.055$) and Methanobrevibacter olleyae ($r = 0.94$, $P = 0.017$) showed strong positive correlations with propionate concentrations, suggesting a mechanistic link between microbial composition and reduced methanogenesis. Overall findings suggest that a moderately shortened fattening period (starting at 13 months, slaughter at 28 months) improved feed efficiency and significantly reduced GHG emissions, while maintaining stable rumen microbial ecosystems, offering a practical strategy for more sustainable Hanwoo beef production.

Isotope patterns in fiber fractions of grass samples with high variability in nitrogen content

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Determining the feed value of dairy-intended feed or predicting ruminal gas production currently necessitates animal-based experiments or at least fistulated ruminants as rumen fluid donors for in vitro incubations. However, considering the 3R strategy (Directive 2010/63/EU), non-invasive modeling approaches might be a promising alternative. However, modeling pathways of microbial fermentation require the absence of differences in the fiber fractions of feed. Therefore, in this study, isotope patterns of varying grassland samples were determined across various crude protein (CP) concentrations. Samples (n = 42) were taken from a long-term field study of permanent grassland at the Technical University of Munich that highly differ due to the long-term fertilization strategy. After gentle drying (60 °C), dry matter (DM), CP (by Kjeldahl N-determination), crude ash (CA), crude fat (CL), and fibre components (neutral detergent fibre (aNDFom), acid detergent fibre (ADFom), and acid detergent lignin (ADLom)) were analysed according to VDLUFA (2023). Additionally, carbon and nitrogen concentrations, as well as $\delta_{13}C$ and $\delta_{15}N$, were measured in the original samples, and their fiber fractions, whereby samples were prepared by boiling in the corresponding neutral or acid solution, and drying them according to the determination of aNDFom, ADFom, and ADLom (VDLUFA, 2023), although ashing was skipped. Carbon, nitrogen, $\delta_{13}C$ and $\delta_{15}N$ were determined by combusting samples in an elemental analyzer coupled to a continuous-flow IRMS. Depending on CP concentration, samples were categorized into high (n = 15, 15.2-18.1% DM), medium (n = 14, 12.23-14.7% DM) and low (n = 13, 8.8-12.1% DM) CP samples, which were used for statistical analyses using a general linear model comparing the CP groups and a mixed model with fiber fractions (original sample, aNDF, ADF, ADL fraction) and CP group as fixed factors. Samples with lower fiber components showed increased CP concentrations ($p < 0.05$), while CA was similar between groups ($p > 0.05$). In the lowest CP group highest C/N ratio was observed and highest CP group had lower $\delta_{13}C$ than the lowest CP group ($p < 0.001$), whereas total carbon and $\delta_{15}N$ were similar across groups. Additionally, all parameters differed significantly between the fiber fractions ($p < 0.001$). I.e., $\delta_{13}C$ was highest in aNDF and lowest in ADL, while $\delta_{15}N$ in ADF was significantly below the remaining fractions. The within-fraction heterogeneity suggests that modeling rumen processes could be more complicated than expected and likely benefits from separate analysis of isotope pattern in the individual feed fractions. The project is supported by funds of the Federal Ministry of Agriculture, Food and Regional Identity (BMLEH) based on a decision of the Parliament of the Federal Republic of Germany via the Federal Office for Agriculture and Food (BLE), grant number 2825ERA02U and as part of the CSA Green ERA-Hub has received funding from the European Union's research and innovation Programme Horizon Europa under grant agreement No 101056828.

Use of essential oils on enteric methane emissions and ruminal microbiota of finishing feedlot Nellore cattle

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Reducing enteric methane (CH_4) emissions from ruminants is a global priority to improve the environmental sustainability of livestock production. Nutritional strategies capable of mitigating methanogenesis without compromising animal productivity are particularly important in tropical beef systems, where information on effective mitigation approaches remains limited. Essential oils (EOs) have emerged as promising natural rumen modulators due to their antimicrobial properties and their capacity to alter microbial fermentation pathways involved in hydrogen metabolism. This study evaluated the effects of dietary supplementation with a plant-derived additive containing carvacrol and capsaicin (ValoPro-E) on enteric methane emissions and rumen microbial community structure in finishing Nellore feedlot cattle under tropical production conditions. Forty-eight Nellore young bulls (initial body weight = 333.6 ± 4.1 kg) were allocated in a completely randomized design with two treatments (n = 24): control (basal diet + mineral premix) and EO supplementation (basal diet + mineral premix + 3 g/animal/day of additive) over a 119 day period. Diets were formulated to meet the requirements for an average daily gain (ADG) of 1.5kg/day, with a forage-to-concentrate ratio of 12:88. Animals were housed in collective pens equipped with automated bunks and scales (Intergado®), allowing individual monitoring of feed intake and body weight. Enteric CH_4 emissions were measured in a subset of animals (n = 12 per treatment) using the sulfur hexafluoride (SF□) tracer technique with continuous 24-h sampling over five consecutive days. Rumen samples (100g) were collected immediately postmortem and placed in insulated boxes at 4° C. The rumen microbiota was characterized by 16S rRNA gene sequencing, and differentially abundant taxa were identified using LEfSe analysis. Dry matter intake and average daily gain were not affect by EO supplementation (9.20 vs 8.99 kg/day and 1.15 vs 1.16 kg/day, respective). EO supplementation significantly reduced CH_4 emissions compared with the control group (174.6 vs. 193.1 g/day; P = 0.0023), representing a 9.5% reduction in enteric methane production and . Microbial community analysis showed that the rumen microbiota was dominated by the bacterial phyla Bacteroidota and Firmicutes in both treatments; however, EO supplementation promoted distinct shifts in microbial composition. Specifically, the abundance of Peptostreptococcus and Anaerobacterium increased with EO supplementation, whereas several carbohydrate-fermenting bacteria and potential hydrogen-producing taxa were more abundant in the control group. These microbial shifts indicate a modification of ruminal fermentation pathways that may reduce the availability of metabolic hydrogen for methanogenesis. Overall, EO supplementation effectively reduced enteric methane emissions and was associated with significant alterations in rumen microbial community structure. These findings suggest that targeted modulation of rumen microbiota represents a promising nutritional strategy for methane mitigation in tropical beef cattle production systems. This study was supported by FAPESP (grants 2017/50339-5, 2021/11922-2) and CNPq.

Effects of condensed tannin extract supplementation on performance, carcass traits, and methane emissions of feedlot cattle fed diets with different protein profiles

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Ruminal protein degradability influences overall diet digestibility, nitrogen excretion patterns, and animal performance. Excess rumen degradable protein (RDP) increases nitrogen excretion, whereas insufficient supply limits ruminal digestion; therefore, a minimum level is required to optimize digestive and metabolic efficiency while maximizing animal performance. Condensed tannin extract (CTE) has the potential to modulate ruminal protein degradability, increasing metabolizable protein supply without impairing ruminal microbial efficiency. In this context, the aim of this study was to evaluate the supplementation of CTE in feedlot containing two protein sources differing in rumen degradable protein (RDP) content on dry matter intake (DMI), animal performance, carcass traits, and methane emissions, hypothesizing an increase in performance along with a reduction in methane emissions. Ninety-six Nellore bulls (375 ± 18 kg of initial body weight) were blocked by initial body weight and assigned within blocks to 24 collective pens (n = 6 pens per treatment). The experimental design used was randomized blocks in a 2 × 2 factorial arrangement where, factor 1: dry distillers grains plus solubles (DDGS) or peanut meal; factor 2: with or without inclusion of CTE from quebracho (Schinopsis sp.). The treatments evaluated were: T1: DDGS (~50% RDP); T2: DDGS + CTE (0.08% of dry matter [DM]); T3: Peanut meal (~65% RDP); T4: Peanut meal + CTE (0.08% of DM). The performance trial lasted 105 days. At the end of this period, 48 bulls were randomly selected within blocks (n = 12 per treatment), individually housed, and evaluated for methane emissions using the SF□ tracer technique over a 25-day period. Data were analyzed using a mixed-effects model, with block and experimental unit included as random effects, and protein source, tannin supplementation, and their interaction included as fixed effects. Differences were considered statistically significant at $P \leq 0.05$. No differences were observed among treatments for DMI ($P \geq 0.079$). Bulls receiving condensed tannins (T2 and T4) showed greater average daily gain (ADG) than non-supplemented treatments (T1 and T3) (1.74 and 1.65 vs. 1.61 and 1.61 kg/d; P = 0.015), resulting in higher final body weight (557 and 549 vs. 543 and 544 kg; P = 0.018) and trend to improve feed efficiency (0.170 and 0.164 vs. 0.157 and 0.164 kg; P = 0.063), regardless of protein source. A significant protein source × tannin interaction was observed for hot carcass weight (HCW; P = 0.050), dressing percentage (DP; P = 0.005), and carcass daily gain (CDG; P = 0.043). Tannin supplementation increased HCW (317.97 vs. 330.04 kg; P = 0.033), DP (57.82 vs. 58.91%; P = 0.024), and CDG (1.24 vs. 1.36 kg/d; P = 0.025) in DDGS-based diets, whereas no improvement was observed when peanut meal was used (322.01 vs. 319.04 kg; 58.17 vs. 57.28%; 1.28 vs. 1.25 kg/d; $P \geq 0.578$). No treatment effects were observed for methane emission parameters ($P \geq 0.161$). Therefore, CTE supplementation enhanced overall performance, with greater benefits on carcass traits when lower RDP diets were fed. The improvements associated with tannin supplementation are likely linked to modifications in ruminal fermentation patterns and the dynamics of nitrogen and energy metabolism.

Does a combination between protein reduction and yeast probiotic in the diet of dairy cow impact feed efficiency, nitrogen use, digestibility and rumen microbial protein synthesis?

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Reducing dietary crude protein (CP) in dairy cows can lower nitrogen (N) excretion and improve N use efficiency (NUE) but may impair ruminal fermentation and productivity. This study evaluated whether reducing protein level alone or in combination with yeast probiotic Saccharomyces cerevisiae CNCM I-4407 (Actisaf® Sc 47) could mitigate these effects. In this study, we evaluated N utilization, feed efficiency (FE), digestibility and rumen microbial protein (RMP) synthesis. Six multiparous Holstein cows (92.5 ± 2.6 DIM; 38.7 ± 5.9 kg milk/d) were assigned to a 3 × 3 Latin square design with three 23-d adaptation periods and 5-d sampling periods, separated by 15-d transition periods. Treatments were: control (CTR; 16.5 % CP in DM), low-CP (LCP; 14.5 % CP in DM), and LCP + Actisaf Sc 47 (5 g/d; LCPActisaf). During the 5 days of sampling, milk samples (100 ml) were individually collected and analyzed for fat and protein to calculate energy corrected milk (ECM) and FE. For NUE, RMP, and digestibility, six spot samples of urine (100 ml) and feces (500 g) were collected at the same time interval over a 24-h cycle. At the end of each sampling period, all the urine samples were mixed, and 30 ml were analyzed for creatinine, N and purine derivatives (PD) to estimate total urine output, NUE and RMP. Offered diets and feces were analyzed for acid insoluble ash (AIA), dry matter, neutral detergent fiber and N to estimate digestibility. Urine output was calculated as the ratio between the creatinine coefficient of 29 mg/kg BW/day and creatinine concentration in urine. RMP was estimated as the ratio of absorbed PD multiplied by the purine protein content (437.5 mg CP/mmol) and divided by the ratio of purine N to total N in mixed rumen microbes (0.116), multiplied by microbial purine digestibility (0.83). FE was calculated as the ratio of ECM to DMI. NUE was calculated as the ratio of N in milk to N intake. Data were analyzed using a generalized linear mixed model including treatment, period and their interaction as fixed effects and cow as a random effect. Cows in the CTR group had numerically greater FE when compared with cows in the LCP group (1.46 vs 1.39; $p = 0.1$) but were similar ($p = 0.39$) to cows in the LCPActisaf group (1.51). Cows in the LCPActisaf group had greater FE when compared with cows in the LCP group ($p = 0.02$). Compared with CTR, N intake was reduced by both LCP and LCPActisaf (−12.95% and −17.44%, respectively; $p = 0.003$). Compared with CTR, fecal N (FN) was not impacted by LCP (233 vs 211.29 g/d; $p > 0.1$), but was decreased by LCPActisaf (182.9 g/d; $p = 0.04$); LCPActisaf tended to decrease FN compared with LCP ($p = 0.1$). Compared with CTR, LCP did not impact urinary N (UN) (264.4 vs 218.3 g/d for CTR and LCP, respectively), however, LCPActisaf tended to decrease UN (211 g/d; $p = 0.1$). NUE tended to increase with LCP and was improved with LCPActisaf (28.3 vs 31.3 vs 35.1 for CTR, LCP and LCPActisaf, respectively; $p = 0.007$). Cows in the LCPActisaf group tended to have greater protein digestibility when compared with LCP (67.9 vs 63.49; $p = 0.1$) but were similar to CTR (67.90 vs 64.25; $p = 0.32$). Despite a greater RMP synthesis in LCPActisaf, no difference was observed between the three treatments (1957 vs 1874 vs 1748 for LCPActisaf, LCP and CTR groups, respectively; $p = 0.95$). In conclusion, reducing dietary CP combined with Actisaf Sc 47 supplementation maintains milk production, enhances FE and NUE, and reduces N excretion, offering a promising strategy for more sustainable dairy production systems.

Testing coated calcium peroxide for methane mitigation in dual-flow fermenters

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There is a growing body of research investigating the use of oxidising compounds, such as calcium peroxide (CaO_2), as potential strategies for methane mitigation in agricultural systems. Methane produced during enteric fermentation in ruminants represents a significant source of greenhouse gas emissions from livestock production. Previous studies have demonstrated that CaO_2 can effectively suppress methane production both in vitro and in vivo in beef cattle without negatively affecting animal health or performance. However, when administered in powder form, CaO_2 reacts rapidly with moisture to produce oxygen (O_2), typically within a matter of hours. This rapid reaction results in only short-term effects, limiting its practical application under grazing conditions. Developing a coated formulation of CaO_2 could therefore provide a controlled, slow-release mechanism, extending its activity in the rumen and improving its suitability as a methane-mitigating feed additive for pasture-based farming systems. The aim of this research was to develop and evaluate a coating strategy capable of slowing the release of CaO_2 within the rumen environment. Initial closed-system batch experiments assessed the performance of a gelatine-coated CaO_2 formulation. These preliminary trials demonstrated a significant reduction in methane production when gelatine-coated CaO_2 was used compared with uncoated CaO_2 ($p < 0.001$), indicating that the coating may enhance the efficacy of the treatment by modifying its release dynamics. Following these encouraging results, a subsequent in vitro experiment was conducted using dual-flow continuous culture fermenters in order to evaluate the coated formulation under more representative rumen simulation conditions. The experimental design consisted of a 14-day acclimation period followed by a 7-day sampling phase. Fermenters were fed daily with a diet consisting of a 1:1 ratio of grass silage and coarse ration at a rate of 10 g dry matter per day. Treatments were applied in triplicate, with three fermenters receiving uncoated CaO_2 and three receiving the gelatine-coated CaO_2 formulation. Following administration of the treatments, oxidative reduction potential (ORP) increased to above +100 mV across all fermenters, reflecting the oxidative effect of CaO_2. However, differences were observed in the rate at which ORP subsequently declined. In fermenters receiving the gelatine-coated treatment, the decrease in ORP occurred more gradually than in those receiving uncoated CaO_2. This pattern suggests that the coating slowed the release of CaO_2, resulting in a more sustained oxidative effect within the system. Overall, these results indicate that coating CaO_2 with gelatine can successfully modify its release profile in continuous dual-flow fermenters, allowing for a slower decline in ORP compared with the uncoated powder form. This supports the potential of coated CaO_2 formulations as a methane mitigation strategy for ruminant production systems. Further in vitro work is required to optimise the coating composition and release characteristics. Future research will also include small-scale animal trials to evaluate the treatment in vivo, with particular attention to methane emissions, nutrient digestibility, and potential impacts on the rumen microbial community.

Nitrogen balance and nitrogen use efficiency in extensive Charolais beef cattle systems facing nitrate water pollution risk

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Nitrogen (N) management is a key challenge for improving the environmental performance of livestock farming systems, particularly in areas vulnerable to nitrate pollution. This study assesses N balance and nitrogen use efficiency (NUE) at the farm scale in Charolais beef cattle systems located in the Charolais-Brionnais region (Saône-et-Loire, France). In 2020, during the seventh revision of nitrate vulnerable zones, 128 municipalities in western Saône-et-Loire were proposed for classification following high nitrate concentrations in surface and groundwater. This decision raised concerns among stakeholders, as the region is dominated by extensive grassland-based livestock systems with low stocking densities (around 1.2 livestock units per hectare) and a high proportion of permanent grasslands, which theoretically limit nitrate leaching risks. Within this context, N flows were quantified in five representative Charolais beef farms. N inputs included mineral fertilizers, purchased feed, animals, and atmospheric N fixation from grasslands, while outputs corresponded mainly to animal products and crop exports. NUE was calculated as the ratio between useful N outputs and total N inputs. Results showed relatively low N inputs typical of extensive grassland systems, ranging from 11 to 79 kg N/ha per year, while N outputs ranged from 6 to 18 kg N/ ha per year across farms. Consequently, N surpluses remained moderate, with farm N balances ranging from approximately 5 to 61 kg N/ ha per year, depending on farm structure and production objectives. Nitrogen use efficiency (NUE) varied considerably among farms, reflecting differences in management practices, herd composition, and input use. In particular, specialized cow-calf systems that showed lower N surpluses than mixed cow-calf and finishing systems. Overall, the results highlight opportunities to improve N management while maintaining the extensive nature of these production systems. The findings contribute to a better understanding of N dynamics in herbivore-based livestock farms and provide scientific evidence to inform regional discussions on nitrate vulnerability classification and the adaptation of N management policies to grassland-dominated livestock systems.

The environmental impact of dairy cattle farming using Life Cycle Assessment: a meta-analysis

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Multiple Life Cycle Assessments (LCA) have been implemented to estimate the environmental footprint of cattle milk. The aim of this study was to collect available LCA studies, streamline their data (harmonise functional units, allocation methods, impact category units, milk yield, herd size and grazing period) and run a meta-analysis to evaluate the environmental impact of milk production at the farm level. A total of 274 studies with 2928 observations were used. A statistical analysis was performed between six parameters (Continent, Climate, State of LCA, Farm system, Number of animals and Milk yield) and five environmental categories (Global Warming Potential – GWP, Acidification Potential – AP, Eutrophication Potential – EP, Non-renewable Energy – NRE and Land Use – LU). The dataset covered dairy LCA studies across the world allowing the presentation of global mean values for the impact categories examined. The mean contribution of dairy farming in the intensity of greenhouse gas emissions was 1.27 ± 1.32 kg CO2eq. kg Fat and Protein Corrected Milk (FPCM)$_{-1}$. In terms of acidification and eutrophication dairy farming contributed 10.60 ± 5.88 g SO2eq. kg FPCM$_{-1}$ and 5.16 ± 2.53 g PO4$_{3-}$eq. kg FPCM$_{-1}$, respectively. Additionally, it used non-renewable sources at a rate of 2.90 ± 1.05 MJ kg FPCM$_{-1}$ and, on average, occupied a land of 1.49 ± 1.69 m$_2$ kg FPCM$_{-1}$ for its production. The LOW milk yield category demonstrated 83 % higher GWP and 138 % higher LU compared with the VERY HIGH category (1.87 kg CO2eq. kg FPCM$_{-1}$ and 2.86 m$_2$ kg FPCM$_{-1}$, for GWP and LU, respectively). In contrast, HIGH and VERY HIGH categories had a significantly higher AP compared with lower yielding categories. Farms with more than 1,001 animals showed the lowest GWP, 27 % lower compared with smallest farms (0.99 vs 1.26 kg CO2eq. kg FPCM$_{-1}$ for more than 1,0001 animals vs less than 50 animals), while farms with 201 to 1,000 heads had an intermediate value. It is noteworthy that farms with a herd size larger than 1,000 heads had overall the lowest values in four out of five impact categories analyzed when compared with other farms. Overall, the current study found that high milk yields, a herd size of over 1,000 animals, temperate and colder climates were linked to low GWP and LU values. Although, except for large herds, the other categories had apparent environmental drawbacks, especially in the AP and EP impacts. In contrast, farms located in regions with developed dairy farming had lower environmental footprints. This study, taking into account a broader environmental scope, demonstrated that the largest farms displayed the lowest emissions per kg of Fat- and Protein- corrected Milk. Results showed that assessing environmental performance based only on GWP without taking into consideration more impact categories could be misleading.

Modeling carbon cycle from dairy cows, barn, and storage

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Inventory calculations for emissions from animals and manure are often based on low-Tier emission models; however, nutrient excretion and emissions depend on animal, diet, barn and manure storage characteristics. Carbon from feed ends up in milk, weight gain, feces, urine and gases (CH4 and CO2). These gases originate from rumen fermentation and respiration from the animal, and from manure fermentation and surface respiration in the barn and storage. The aim of the present study was to model carbon cycling at cow, barn and storage level based on dry matter intake (DMI), diet composition, nutrient digestibilities, performance level and farm management. The data were modelled for a dairy farm with 280 cows [11 tons milk/cow/y and 23 kg DMI/cow/d], representing milk yield and feed intake of a typical Danish dairy farm. Slurry emissions were modelled based on 28 d removal interval from a barn with a ring-channel slurry storage under a slatted floor. Slurry was stored in an outside storage with a natural crust (mean retention time 2.8 months). Enteric methane was estimated based on DMI and diet composition (Hellwing et al., 2016). Nutrient composition of feces was calculated based on feed composition and digestibilities of key nutrients. Methane emission from slurry was estimated with the anaerobic biodegradation model (ABM), which was based on hydrolysis of organic matter (OM) to volatile fatty acid (VFA) and then VFA conversion to CH4 and CO2. Two diets with 60% forage (80:20 or 20:80% of grass-clover to corn silage, GS and CS, respectively) were modelled. Carbon intake (kg/d) was 9.3 in GS and 9.2 in CS. Carbon excretion (% of intake) for GS and CS diets was similar in milk (22), CO2 (41), and feces (31). Carbon (% of intake) in enteric CH4 was higher in GS (3.4) than in CS (3.2). Carbon (% of intake) in urine compounds was higher in GS (1.3) than CS (1.0). Carbon excreted in slurry (feces+urine+2% of DMI feed spill) was the same in GS and CS (3.2 kg/d). At barn level, carbon (% of carbon excreted in slurry) in CH4 was higher in CS (3.4) than in GS (3.1), while CO2 was higher in GS (10.7) than in CS (10.1). At storage level, carbon (% of carbon excreted in slurry) in CH4 was higher in CS (2.3) than in GS (1.9), and the CO2 was the same across diets (4.6). Earlier studies found that diets resulting in lower enteric CH4 emissions may result in higher emissions from slurry. This can potentially be explained with lower digestibility of nutrients, resulting in more degradable OM in the slurry, or a different chemical composition of manure excreted, e.g. a higher share of undigested starch, degradable fibers or fat. When evaluating dietary mitigation strategies like fat supplementation, downstream emissions should be included in decision making. In conclusion, the current model shows that it is possible to combine models for nutrient digestion and enteric emissions from animals with degradation in barn and manure storage by including details on nutrient composition and carbon flows, offering a broader perspective on emissions from dairy farms than enteric fermentation alone.

Temperature-driven seasonal variation of soil CO_2 and N_2O emissions under rotational and continuous grazing in a Mediterranean grassland

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Accurate greenhouse gas (GHG) inventories for Mediterranean pastoral systems are often confounded by the synergistic effects of seasonal thermal stress and grazing-induced changes to soil microclimates. This study was conducted at the experimental farm, the University of Tuscia (Viterbo, Central Italy). It sought to decouple the influence of grazing regimes; Continuous (CG) and Rotational (RG) using 18 Sopravvissana sheep per system at a stocking rate of 100 sheep ha□¹ (2 h grazing day⁻¹), with contrasting stocking densities (100 sheep ha□¹ in CG vs. 600 sheep ha□¹ in RG), on soil CO2 and N2O emissions across seasonal transitions from spring to summer. We quantified soil CO2 and N2O fluxes at high temporal resolution using automated chambers coupled with laser absorption spectrometers (LI-7810 and LI-7820, LI-COR Biosciences). To capture peak diurnal variability, flux measurements were strategically stratified into morning (08:00–11:00) and afternoon (15:00–18:00) windows over two consecutive days, ensuring representativeness of the mediterranean microclimate. This synchronized, high-frequency monitoring allowed for the detection of gas concentration shifts across ryegrass–clover (spring trial) and teff (summer trial) phenological stages. Soil temperature and moisture were recorded using a Soil Hydra probe installed at 5 cm depth. We utilized generalized linear models (GLMs) and segmented regression to identify environmental breakpoints and management-driven substrate legacies. Soil temperature emerged as the main predictor for both gases ($p < 0.001$). While pre-grazing CO2 fluxes were uniform, post-grazing RG showed significantly higher emissions (8.34 ± 0.22 vs. 7.36 ± 0.19 µmol m^{-2} s^{-1}) compared to CG in spring, suggesting that pulsed grazing under rotational management accelerates root exudation and microbial respiration during regrowth phase. N2O emissions did not differ significantly between grazing systems in spring trial. Conversely, the pre-summer stage revealed a "legacy effect" in CG, which exhibited significantly higher CO2 (15.17 ± 4.49 vs 10.01 ± 2.61 µmol m^{-2} s^{-1}) and N2O (0.12 ± 0.09 vs 0.05 ± 0.03 nmol m^{-2} s^{-1}) emissions than RG ($p < 0.05$). This indicates that sustained grazing pressure may accumulate labile substrates that become highly reactive during early summer warming. Segmented regression identified a critical temperature threshold at approximately 28°C to 30°C. Beyond this point, N2O flux sensitivity increased non-linearly, signaling a potential shift in microbial nitrogen transformation kinetics. Our findings demonstrate that grazing impacts are not static but are highly modulated by seasonal thermal dynamics. The temperature threshold points for N2O suggest that Mediterranean systems may face disproportionate emission spikes under climate warming scenarios. These findings underscore the necessity of seasonally adapted management frameworks. Specifically, strategical timing rotational rest periods to coincide with critical thermal transitions can significantly mitigate the carbon and nitrogen footprints of Mediterranean pastoral systems.

Mitigating emissions from manure: The effectiveness of biochar under real dairy farm conditions

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Livestock housing is a significant source of ammonia (NH3) and non-CO2 greenhouse gas emissions due to volatilization from freshly excreted manure. Mitigation strategies applied directly at the emission source may reduce these losses. This study evaluated the effectiveness of biochar as a manure amendment for reducing gaseous emissions under real dairy barn conditions. The experiment was conducted during winter 2025 at the Agricultural Research Institute (ARI) experimental dairy farm in Cyprus using lactating Holstein cows housed in a free-stall barn. All cows received the same diet under standard farm feeding conditions, consisting of 15–17 kg of concentrated feed pellets (19% crude protein) via automatic feeders and 9–10 kg dry matter (DM) of hay and silage per cow/day.Two comparable groups of 12 cows were maintained in separate barn sections, where one group received bamboo biochar (Phyllostachys aurea) application (2.5% w/w, dry matter basis) directly to freshly excreted manure, while the other served as an untreated control. Fresh manure was collected daily from different parts of each barn, pooled and transferred into replicated chambers (six per treatment) for real-time gas emission measurements. No manure removal or cleaning was performed in the barn during the experimental period, while only the manure in the chambers was replaced every 24 h with newly collected material over a 10-day monitoring period. Gas fluxes (NH3, CO2, CH4, and N2O) were measured using automated non-steady-state chambers coupled with a cavity ring-down spectroscopy analyzer, providing high-frequency measurements (about 16 measurements per chamber per day) and enabling detection of short-term emission dynamics.The results showed that biochar application substantially reduced cumulative NH3 emissions by 58%, decreasing losses from approximately 8.2 to 1.5 g m-2. Temporal analysis revealed that mitigation occurred through a gradual flattening of NH3 emission trajectories rather than immediate suppression. Methane emissions exhibited a moderate cumulative reduction (approximately 49%), although no treatment effect was observed at the daily scale. In contrast, cumulative CO2 emissions were higher under biochar treatment, while N2O emissions remained very low and unaffected.Although measurements were conducted in chamber systems, manure was sourced directly from treated and untreated barns and renewed daily in the chambers, ensuring that emission patterns reflect practical barn conditions during the immediate post-excretion phase.

Effect of ventilation regimes on carbon and nitrogen dynamics in composted bedded packs for Mediterranean dairy cattle production

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Composted bedded packs in dairy systems are strongly influenced by environmental conditions. In warm Mediterranean climates, mechanical ventilation may play a key role in bedding nutrient dynamics, particularly nitrogen (N) and carbon (C). This study evaluated the effect of different ventilation regimes on physicochemical properties and N and C availability in a commercial dairy farm in eastern Spain. The trial was conducted from January to March 2025 on a commercial farm in Valencia (Spain). Three ventilation strategies were compared: (i) no mechanical ventilation, (ii) temperature-controlled ventilation, and (iii) continuous ventilation at full capacity during a fixed daily time slot. Eight weekly samplings were performed in four pens, collecting composite samples at the surface and 15–20 cm depth. Across all treatments, deep bed temperatures remained low (16 °C), well below the optimal range for active composting (45–55 °C). N concentrations were high (3%) and C levels moderate (38%), resulting in low C:N ratios (13:1), far from the optimal 25:1–30:1 range. These conditions likely limited microbial activity, reducing C stabilisation and altering C:N dynamics. High pH values (>9) and the absence of an effective thermophilic phase may have further constrained microbial N immobilisation, maintaining N in more reactive forms. The lack of active composting could be associated with altered N losses and reduced C stabilisation, with potential implications for greenhouse gas emissions. Overall, the evaluated ventilation regimes and management practices during the January–March observation period did not achieve conditions suitable for active composting, thereby influencing C and N dynamics. While these results are specific to the study period, they suggest that optimising ventilation and bed management strategies may be necessary in Mediterranean dairy systems, although further research is required to confirm their representativeness across the full annual cycle.

Interaction between protein source and condensed tannin extract on nitrogen metabolism in feedlot cattle

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Improving nitrogen use efficiency (NUE) in feedlot systems remains a challenge, particularly when high rumen degradable protein (RDP) diets increase ruminal ammonia production and urinary nitrogen losses. Protein sources differing in rumen degradability may interact with condensed tannin extract (CTE), altering NUE. Thus, this study evaluated the effects of CTE supplementation in diets containing two protein sources differing in RDP content on nitrogen intake, excretion, retention, and NUE in feedlot cattle. Eight ruminally cannulated Nellore steers (408 ± 41 kg body weight [BW]) were individually housed and allocated to a replicated 4 × 4 Latin square design, containing four periods of 21 d and a 5-d washout interval. Treatments were structured in a 2 × 2 factorial arrangement, consisting of two protein sources (dry distillers grains plus solubles (DDGS) or peanut meal) and with or without CTE from quebracho (Schinopsis sp.). Diets contained 27% roughage and 73% concentrate, were isonitrogenous (14% CP) and isoenergetic (1.33 Mcal/kg NEg) and differed in RDP content (50% for DDGS-based diets and 64% for peanut meal-based diets). The treatments evaluated were: T1: DDGS; T2: DDGS + CTE (0.08% of dry matter [DM]); T3: Peanut meal; T4: Peanut meal + CTE (0.08% of DM). The trial lasted 105 days. Animals were fed ad libitum, and dry matter intake (DMI) was recorded daily as the difference between feed offered and refusals collected the following day. On days 17, 18, and 19 of each experimental period, samples of the diet and orts were collected, and spot samples of feces and urine were collected twice daily; all samples were pooled to form a composite sample for each steer. Creatinine was used to estimate total urinary volume, and acid detergent insoluble ash was used as an internal marker to estimate fecal output. Nitrogen balance was calculated as nitrogen intake minus nitrogen excretion in feces and urine, and NUE was calculated as nitrogen balance divided by nitrogen intake. Additionally, urinary urea excretion was quantified. Data were analyzed using a mixed-effects model, including Latin square, animal nested within Latin square, and experimental period as random effects, and protein source, tannin supplementation, and their interaction as fixed effects. Effects were considered significant at $P \leq 0.05$. No differences were observed among treatments for DMI or nitrogen intake ($P \geq 0.122$). However, a main effect of protein source was observed for urinary nitrogen excretion, which was lower in steers fed DDGS compared with peanut meal (82.01 vs. 91.69 g/d; $P = 0.002$). Additionally, CTE supplementation reduced urinary nitrogen excretion (83.01 vs. 90.69 g/d; $P = 0.010$). Accordingly, steers fed DDGS excreted less urinary urea than those fed peanut meal (107.10 vs. 129.17 g/d; $P < 0.001$). In addition, CTE supplementation reduced urinary urea excretion compared with the treatments without CTE (112.18 vs. 124.08 g/d; $P = 0.022$). In contrast, fecal nitrogen excretion was not affected by either protein source or CTE supplementation ($P \geq 0.274$). Nitrogen retention and NUE did not differ among treatments ($P \geq 0.227$). In this context, beyond reducing urinary nitrogen losses, CTE supplementation may lower hepatic urea synthesis, thereby decreasing the energetic cost of nitrogen metabolism and potentially improving animal performance and environmental outcomes.

Low crude protein diet balanced for metabolizable methionine positively impacts performance, metabolic status and N emissions in high-yielding dairy cows

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Protein efficiency is important to the economic and environmental sustainability of modern dairy production. Feeding high-protein diets to dairy cows leads to excess nitrogen (N) excretion that contributes to the farm environmental footprint. In addition, over-feeding expensive protein sources is economically unprofitable and does not benefit production performance. Reducing dietary crude protein (CP) is a viable environmental and economic strategy, but most often requires essential amino acids supplementation to prevent performance losses. Such feeding strategy is increasingly applied after the peak of lactation, because of a general reluctance to reduce the dietary protein level in early lactation stage. Therefore, this study aimed to investigate the effects of reducing dietary CP in combination with rumen protected methionine (RP-Met) supplementation on performance, metabolic status and urinary N excretion of early lactating dairy cows. The study was conducted on a commercial dairy farm in South Germany over 12 weeks of trial period. A total of 52 peripartum Holstein cows (18 primiparous and 34 second-lactation) were divided in two experimental groups, fed either a high-CP (16.9% DM; CON) or a low-CP (15.8%; RPM) diet supplemented with 15 g/day/cow of RP-Met (Mepron®, 85% DL-methionine, Evonik Industries, Germany). Individual milk yield (MY) was recorded daily and milk composition was analyzed every two weeks. Backfat thickness was measured biweekly by ultrasound (EasiScan, IMV-Imaging) at a caudal distance of about 10 cm from Tuber ischiadicus towards Tuber coxae. Blood samples were taken from the caudal vein (2 weeks prepartum and 0, 7, 14 and 50 days in milk) and analyzed for β-hydroxybutyrate (BHB) and urea-N content. Urinary N excretion was quantified during the last trial week, using creatinine concentration as an indicator of urine volume. Dry matter intake (DMI) was registered daily at group level. Statistical effects were analyzed based on a linear model using treatment and period (week) and their interaction as fixed effects. Least square means were compared using Tukey's test. Significance was declared when $P \leq 0.05$ and tendency with $0.05 < P \leq 0.10$. Dry matter intake was not affected by the reduction of dietary CP (24.4 and 24.6 for RMP and CON, respectively). Milk yield tended (P=0.054) to increase in the RPM group (43.8 and 40.5 kg/d for RPM and CON, respectively), while milk fat and protein content were not affected. Blood and milk urea-N were significantly lower in the RPM group after 14 and 50 days, indicating a more efficient N utilization. Urinary N excretion was significantly reduced (P=0.021) with RPM when compared to CON (164 and 218 g/d, respectively). BHB levels for both, CON and RPM, were within the physiological range at any time. Nevertheless, the backfat thickness curve of RPM indicated less fat mobilization than in CON. Contrary to CON, RPM already gained weight from about 50 DIM. In conclusion, the reduction of dietary CP combined with RP-Met supplementation proves to be a practical solution for improving N efficiency and metabolic status in dairy cows. In addition, N losses to the environment are reduced without compromising milk production performances.

Multifunctional utilisation of dual-purpose cropping for pasture, forage & grain

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The European livestock sector is increasingly concentrated in specific regions with limited integration between animal and crop production. High livestock densities generate substantial local environmental impacts and increase the risk of nutrient leakage. In contrast, arable regions with limited livestock production often experience shortages of nutrients and depend heavily on mineral fertilizers. Improved integration of crop and livestock production could provide both economic and environmental benefits by strengthening circular nutrient flows and resource efficiency in agricultural systems (Wilkins, 2008). Increasing climate variability also requires robust and flexible feed production systems, as livestock depend on reliable access to feed and pasture regardless of yearly growing conditions. Diversification of crop rotations has been shown to increase agricultural resilience to adverse weather and climatic variability (Bowles et al., 2020). One strategy to enhance diversification and crop–livestock integration is the use of dual-purpose crops, where crops are cultivated for more than one purpose, typically forage and grain, allowing flexible use for grazing, silage, or grain depending on seasonal conditions. We hypothesize that combinations of spring and winter annual crops, grown either as primary or undersown crops and harvested for pasture, forage, and grain, can increase feed availability and reducing production risks for dairy and beef producers and minimizing environmental impacts. Dual-purpose cropping systems are well established in semi-arid regions such as Australia and North America (Bell et al., 2015) but remain poorly evaluated in temperate high-latitude environments. Within the Mulcropa project (2024–2027), field experiments have been established at four locations in Sweden representing contrasting temperate climatic conditions at high latitudes. Regionally adapted combinations of spring and winter annual crops are evaluated within crop rotations designed to maximize the growing season while maintaining plant cover and improving nutrient retention. The systems include two-year winter rye, grazed in the first year and harvested for grain in the second year, and maize combined with undersown crops to improve feed production and nutrient cycling. The objective is to evaluate the potential of these crop combinations to simultaneously produce forage and grain while improving resilience and nutrient use efficiency in crop–livestock systems. Agronomic performance, biomass production, feed value, and estimated livestock production potential are presented under varying climatic and growing conditions. The results from the first cycle of the evaluation of the agronomic performance tells that the combinations with main and undersown crops produces equal or better compared to the when crops grown alone in sequence in a rotation. References Bell, L.W., Harrison, M.T. & Kirkegaard, J.A. 2015. Crop & Pasture Science 66:i–iv.Bowles, T.M. et al. 2020. One Earth 2:284–293.Wilkins, R.J. 2008. Philosophical Transactions of the Royal Society B 363:517–525.

Innovation in the U.S. Beef Value Chain: A Sustainable Beef Research Roadmap

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The U.S. beef industry has a long history of improving productivity and environmental performance; however, persistent scientific gaps and increasing demands for credible, scalable sustainability solutions underscore the need for a coordinated national research agenda. This work synthesizes current scientific knowledge and expert input to define a unified research roadmap aligned with the U.S. Roundtable for Sustainable Beef's environmental indicator goals. The roadmap identifies six critical research areas—grazing management plans, feedyard optimization, technology innovation, advanced modeling, genetic improvement, and producer practicality— and 22 key research questions that collectively represent the most influential levers for measurable, scalable improvement. Key opportunities include strengthening the accuracy, consistency, and scalability of measurement approaches for emissions, soil carbon, water use, and ecosystem services; validating emerging technologies such as methane-mitigating additives, remote sensing tools, and virtual fencing; refining mechanistic and ecosystem-level models to better inform decision-making; and expanding phenotyping to enhance genetic selection for efficiency and environmental outcomes. A cross-cutting emphasis on producer adoption and economic feasibility underscores the need for research that is practical, regionally relevant, and capable of driving sustained implementation across diverse production systems. By outlining clear research priorities and high-impact opportunities, this work provides a strategic foundation to guide investment, enhance data credibility, support producer-level decision-making, and accelerate continuous improvement throughout the beef value chain. Keywords: sustainable beef, grazing management, greenhouse gas emissions, water resources, land stewardship, environmental modeling, genetics, producer adoption

Optimal deployment of genetic innovation is essential to increasing the productivity and sustainability of global cattle production systems

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Genetic improvement programs for beef and dairy cattle are necessary but not sufficient for ensuring increasingly sustainable food supply chains around the world. While global population growth is expected to slow by 2050, the demand for animal proteins continues to increase and livestock production remains a core part of regional strategies to meet human nutritional needs, improve livelihoods and ensure food security. Genetic improvement has been, and continues to be, key to widespread gains in the efficiency, productivity and sustainability of cattle production systems. Selective breeding is only the first step, however, and better use of existing herd-level tools, combined with further innovation in genetic management, will be needed to strategically allocate genetic resources and sustainably meet the need for more high-quality protein. Innovative approaches can take any number of forms but may include key enabling technologies such as advanced reproductive management, enhanced phenotype recording, and targeted selection or precision breeding, strategies that are designed to optimize on-farm performance resulting from livestock genetic improvement programs. Implementation of genetic innovation at herd level leads to better outcomes for individual producers, while benefits are aggregated across farms and represent a holistic solution for improving systemwide efficiency and productivity. Enabled genetic improvement has delivered better health, more productivity, enhanced profitability and reduction of environmental impact, which all contribute to more sustainable beef and dairy production systems. Opportunities to accelerate this progress with climate smart interventions are now emerging. Ultimately, the optimal solution will be highly dependent on the production situation, but genetic innovation has the unique advantage of being deployable and scalable across production systems, markets and countries, which will be critical to meeting the challenge of profitably increasing animal protein availability while minimizing environmental impact.

Synergistic modulation of ruminal kinetics and metabolic efficiency by Lithothamnium calcareum and oregano extract: A holistic framework for sustainable bovine production

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The global dairy sector faces an urgent mandate to synchronize intensive production with rigorous environmental stewardship. In this regard, rumen function and animal health have a key role to play. In dairy herds, SmartMag (SM) acts as a superior ruminal buffer compared to traditional sodium bicarbonate; data indicates that SM effectively stabilizes ruminal pH, leading to increases in energy-corrected milk (ECM). This stabilization fosters a robust environment for rumen microbiota, enhancing fibre digestibility and specifically increasing milk yield (kg/d) during peak lactation keeping production persistency. Concurrently, oregano extract (OE) serves as a potent metabolic modifier and its supplementation in dairy cows has demonstrated an increase in production and therefore improving feed conversion ratios. This response is attributed to OE's ability to promote rumen papillae development, increasing papillae length which optimizes nutrient absorption and systemic nitrogen (N) utilization. Beyond performance, this holistic approach provides critical environmental mitigation. Several studies have revealed that OE can reduce enteric methane production by up to 22% by selectively suppressing the absolute abundance of methanogenic archaea without compromising beneficial bacterial populations. Furthermore, OE enhances systemic resilience, significantly increasing serum immunoglobulin (IgA and IgM) concentrations, thereby reducing the metabolic cost of inflammation. This study evaluates a novel nutritional framework integrating Lithothamnium calcareum (SmartMag) and Oregano Extract (PhytaBoost) to optimize ruminal kinetics and metabolic partition. Utilizing a longitudinal, paired-sample statistical design across seven diverse commercial dairy operations (n=7) in Spain, we quantified the impact of this synergistic intervention on bio-economic efficiency. Statistical analysis via Paired-Samples T-test revealed a highly significant elevation in ECM, which rose from a control mean of 38.16 kg/d to a trial mean of 41.29 kg/d ($\Delta = +3.13$ kg/d; $p=0.002$), signifying superior metabolic conversion of dietary inputs. This gain was supported by a significant improvement in feed efficiency, increasing from 1.51 to 1.65 ($\Delta = +0.14$; $p = 0.013$), and a notable rise in milk protein from 3.34 to 3.47 ($\Delta = +0.13\%$; $p=0.016$). The latter indicates a profound optimization of N use efficiency; by capturing more dietary nitrogen into milk solids, the framework mitigates precursors for ammonia (NH3) emissions. Furthermore, a trend in milk fat was observed, increasing from 3.67% to 3.81% ($\Delta = +0.14\%$; $p = 0.074$), while methane intensity trended downward, suggesting a bioactive-driven shift toward propionogenesis over methanogenesis. Economically, the intervention yielded a significant rise in Income Over Feed Cost, increasing from a control mean of €13.44/cow/d to €14.82/cow/d (Δ = +€1.38/cow/d; $p = 0.011$). These findings demonstrate that the SmartMag-PhytaBoost framework provides a scalable, scientifically validated mechanism for sustainable intensification, aligning farm-level profitability with global net-zero and nitrogen mitigation targets. Moreover, our findings suggest that the combination of LC and OE provides a robust nutritional framework to adapt bovine systems to climate stressors, meeting global demands through enhanced feed-to-protein conversion.

Environmental, economic, and technical performance of goat farming systems compared to cattle and sheep systems: A systematic review and meta-analysis across climatic regions

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Livestock production systems face multiple challenges, including low productivity, a growing demand for animal-source foods, and a progressive scarcity of production resources, all of which are further exacerbated by climate change. In this context, goat farming is often considered a particularly relevant option to address these challenges. Scoping literature reviews highlight several advantages associated with goats, which are frequently described as better adapted to heat stress, forage scarcity, and degraded rangelands. However, empirical studies on the potential zootechnical, economic, and environmental advantages of goats relative to other livestock species have yielded mixed results. Moreover, studies examining the potential advantages of goats over other livestock species are often exploratory and largely rely on qualitative assessments. This study aims to provide a quantitative synthesis of the available scientific evidence to robustly assess whether goat farming systems have effective advantages over cattle and sheep systems. A systematic literature review and a meta-analysis were conducted, covering approximately 600 publications from 50 countries over the period 1980-2025. The analysis relies on key indicators describing the zootechnical, socio-economic, and environmental performance of the different livestock systems. The review highlights consistent differences in system performance across livestock species and climatic regions. Goat systems demonstrated superior feed efficiency compared to cattle and sheep in both subtropical (DMI_milk: −39.8% and −45.9%, respectively) and temperate climates (−68.9% and −55.5%), while relying less on grass-based resources across both regions (Grass%: −38.2% to −20.9% vs. cattle; −19.9% to −16.3% vs. sheep). In temperate climates, goat systems emitted less greenhouse gases per liter of milk than cattle (−10.7%) and sheep systems (−50.5%). At the system level, goat systems consistently showed lower GHG emissions than cattle in subtropical (−60.0%) and temperate climates (−62.3%), with more moderate differences relative to sheep (−11.2% and −13.8%, respectively). Regarding economic performance, goat systems showed higher productivity per hectare than cattle and sheep in subtropical regions (+100% and +45.9%), whereas in temperate climates productivity was lower than cattle (−15.9%) but substantially higher than sheep (+81.5%). Production costs per hectare were lower for goat systems than cattle in both climatic regions (subtropical: −20.6%; temperate: −18.2%), and lower than sheep in subtropical contexts (−50.1%), while being markedly higher in temperate settings (+100%). Overall, these results confirm that goat systems offer competitive environmental and economic advantages, particularly under resource-limited or subtropical conditions, although performance remains strongly dependent on climatic region and production context.

Sustainable ruminant livestock transformation through the Global Farm Platform: The hub-and-spoke network

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Ruminant livestock systems provide high-quality nutrition and essential socio-economic services but face mounting challenges from climate change, biodiversity loss, soil degradation, and resource scarcity amid rising global food demand. The Global Farm Platform (GFP), launched in 2014 and honoured by the FAO for excellence in sustainable livestock transformation, offers a proven solution through its hub-and-spoke network. Research farms function as "hubs", living laboratories developing innovations, while commercial farms and smallholders act as "spokes" adapting and scaling solutions in diverse contexts via bidirectional knowledge exchange.This presentation introduces the August Special Issue of Animal Frontiers that showcases ten papers from GFP hubs across five continents. The hub-and-spoke approach rejects one-size-fits-all approaches, instead fostering context-specific, multi-actor innovations that enhance productivity, resilience, and public goods while empowering farmers as co-innovators.Key scientific enablers include adaptive genomics, demonstrating how native breeds such as Vechur cattle harbour adaptive alleles and haplotypes for heat tolerance, disease resistance, energy efficiency, and rumen stability. Using the G→P→E→SI framework and haplotype introgression, these genetic resources support low-input, climate-resilient, low-emission circular systems.Long-term agroecosystem research provides robust evidence: the Pioneer Farm (USA) hybrid hub-and-spoke platform undertakes edge-of-field monitoring and farmer partnerships to resolve soil health and nutrient loss dynamics under real-world conditions. Digital innovation further accelerates progress: practical implementation of FAIR data principles is applied at Harper Adams Future Farm (UK), while advanced digital tools and intensified grazing management for rangelands and native grasslands are utilised in Scotland and Uruguay.Regional applications highlight tangible outcomes: in Africa, the hub-and-spoke model mobilises native forage biodiversity to simultaneously boost productivity, restore soils, regenerate ecosystems, and support smallholder livelihoods. At Silent Valley Farm, India, integrated dairy systems with automated heat-stress mitigation (ASWASA), carbon farming, and native breed conservation deliver "Clean, Green and Ethical" production while strengthening community resilience. In Uruguay, INIA researchers show that long-rotation integrated crop-livestock systems achieve the best sustainability balance, with the highest gross margin, lowest emissions and synthetic N per US$ of margin, and soil erosion well below tolerance limits (compared to continuous cropping). Finally, the UWA Future Farm in Australia demonstrates a holistic multidisciplinary sheep-crop system integrating novel indigenous shrubs, conservation cropping, and community initiatives for Mediterranean environments.Collectively, the papers illustrate that the GFP hub-and-spoke network generates foundational science, translates it into context-specific innovations, and scales solutions effectively. This approach delivers measurable gains in productivity, climate resilience, soil health, biodiversity, and livelihoods while reducing environmental footprints. The GFP offers a scalable, inclusive blueprint aligned with FAO's sustainable livestock transformation vision, the Sustainable Development Goals, and the Paris Agreement.By strengthening such collaborative networks, the livestock sector can produce more nutritious food with less impact, ensuring long-term viability for both planetary and human health.

Grazing management intensification increases pasture height and carrying capacity in native grassland livestock systems: early results from a long-term farmlet experiment

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Native grasslands of the South American Campos biome support extensive livestock systems that deliver food production together with key ecosystem services. However, limited empirical evidence exists on how long-term grazing management strategies influence productivity, resilience, and sustainability at the scale of real livestock systems. This study evaluates the early system trajectories generated by increasing grazing management intensity in native grassland-based mixed livestock production systems. The GLENCOE long-term experimental platform was established in 2023 in northern Uruguay using a farmlet design with three independent 50-ha livestock systems differing only in paddock subdivision: low intensity (2 paddocks), intermediate intensity (8 paddocks), and high intensity (32 paddocks). All systems started with identical vegetation conditions and stocking rates (0.75 livestock units/ha) and were managed under a common adaptive decision framework based on pasture height, animal body condition, and seasonal climate outlooks. Each system integrates beef cow–calf production and lamb finishing under mixed grazing. During the first three years of evaluation, clear differences in system trajectories emerged among grazing intensities. The high-intensity system consistently maintained greater pasture height across seasons, reaching values frequently above 10 cm during favorable growth periods compared with approximately 8–9 cm and 6–8 cm in the intermediate and low-intensity systems, respectively. This enhanced capacity to capture and regulate forage growth enabled progressive increases in stocking rate, reaching approximately 1.08 livestock units/ha in the 32-paddock system compared with 1.04 and 0.91 livestock units/ha in the intermediate and low-intensity systems. Despite these increases in carrying capacity, animal body condition, reproductive performance, and wool production remained comparable among systems. In addition to productive responses, the platform integrates the evaluation of ecosystem services, including soil carbon dynamics, soil biological activity, water quality, plant biodiversity, and ecosystem integrity, together with indicators of animal performance, economic outcomes, and labour conditions. These early results indicate that greater spatial and temporal control of grazing can improve forage capture, increase carrying capacity, and enhance management flexibility in native grassland livestock systems. The GLENCOE platform provides a unique long-term experimental framework to evaluate how grazing management intensification shapes productivity, resilience, and ecosystem services in subtropical grassland biomes.

Summer-smart strategies for livestock production in temperate zones: impact of shade on performance of grazing cattle

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Strategies associated with animal adaptability, resilience and resistance to hotter conditions become more important in a climate change scenario. The most cost-effective environmental modification to ameliorate detrimental effects of heat stress in cattle are shade structures. There is no doubt about the necessity of shade in tropical regions. However, some producers are reluctant to provide shade for livestock in temperate zones as they argue a decrease in animal performance due to the time that cattle spend in the shade instead of grazing. The objectives of this study were to characterize the meteorological conditions during summer in the 'Palo a Pique' long-term research platform located in a temperate region in eastern Uruguay (33.14° S, 54.15° W) and determine the effect of providing artificial shade on average daily gain (ADG) of 15 months old grazing Aberdeen Angus x Hereford steers (mean ± SEM: 303 ± 23 kg liveweight). The temperature-humidity index (THI) was recorded hourly during nine summers using automatic sensors (2007, 2009, 2011, 2013, 2021-2025). Conditions for heat stress were considered thermoneutral when THI < 74, alert (74 ≤ THI < 79), danger (79 ≤ THI < 84), and emergency (THI ≥ 84). In six summers, an independent controlled experiment was carried out in a completely randomized design with two treatments: control steers without access to shade (CONT) and steers with free access to shade in the grazing paddock (SHADE). Three experiments were conducted on Sorghum bicolor var. sudangrass (9.8% CP; 40% ADF; 63% NDF), while the other three experiments were conducted on native grasslands (6.7% CP; 45% ADF; 67% NDF). Stocking rates were 5.8 and 1.7 steers per ha for sudangrass and grasslands, respectively. Shade was provided by a black cloth (4 m2/animal) intercepting 80% of solar radiation mounted 4 m above the ground. A combined analysis of the six independent experiments was carried out using a linear mixed model with treatment as fixed factor and year as random effect. Across summers, mean THI (± SEM) was 70 ± 0.3. Cattle were exposed to the 'normal' category during 67 ± 1.5%, followed by "alert" (23 ± 0.9%), "danger" (9 ± 0.9%) and "emergency" (1 ± 0.5%) categories for heat stress. SHADE steers had higher ADG than CONT steers (0.63±0.10 and 0.54±0.11 kg/a/d, respectively (P < 0.05). As a result of the additional ADG due to the provision of shade (+0.09 kg/a/d), shaded steers would be 8.1 kg heavier than unshaded steers at the end of summer (90 days). Numerically, in none of the six experiments the ADG of CONT animals was greater than the ADG of SHADE animals. Based on the standard nutritive value of beef meat and considering a carcass yield of 52% at slaughter and that 63% of the carcass is available for retail products providing shade would allow the production of additional 6.7 Mcal of energy and 687 g of protein per steer during summer. By combining data from independent trials, which addresses the variability of treatment differences across several summers, we demonstrated that providing shade improved performance of grazing steers in a subtropical zone. This may be an important incentive for shade adoption by farmers as a strategy to improve farm income and animal welfare while contributing to food security objectives.

Dairy Soil and Water Regeneration Research: Filling Crucial Data Gaps

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The University of Wisconsin–Platteville (UWP) Agroecosystems Research Program is conducting an eight-year Dairy Soil and Water Regeneration (DSWR) research project at Pioneer Farm to evaluate the environmental performance and climate resilience of dairy forage production systems. The project is led by Dairy Management Inc. and the Soil Health Institute and includes multiple research institutions, including the USDA Agricultural Research Service and seven university partners located across major dairy-producing regions of the United States. The research aims to identify dairy production systems that maintain high forage productivity while reducing environmental losses and improving the climate-smart performance of livestock systems. At UWP, three dairy-focused crop production systems are being evaluated using a replicated watershed experimental design (n = 4). The first system, a Soil Health Management System (SHMS), includes corn silage fertilized with surface-applied flocculated manure solids, supplemental nitrogen applied according to soil nitrogen tests, and cover crops established immediately following harvest. The second system, Business as Usual (BAU), represents common regional practices in which corn silage is fertilized with fall-injected liquid dairy manure and additional nitrogen is supplied through spring urea applications incorporated by disking, with no cover crops. The third system, Managed Intensive Grazing (MiG), consists of forage-legume-forb pasture mixtures rotationally grazed by bred heifers or dry cows for short grazing periods followed by rest intervals to allow adequate regrowth. Each watershed is instrumented with meteorological stations and soil sensors to monitor air temperature, rainfall, soil temperature, soil moisture, and soil oxygen. Edge-of-field surface-water discharge is measured year-round using H-flumes equipped with ultrasonic stage sensors. Flow-weighted composite samples are collected during runoff events and analyzed for sediment and nutrient parameters including nitrogen and phosphorus species. Surface-water quality is further evaluated using rainfall simulations conducted on small plots (1 m × 2 m) with rainfall intensity of 2.75 inches per hour to replicate high-intensity precipitation events expected to increase under future climate conditions. Complementary monitoring of vadose zone leachate is conducted using undisturbed soil core lysimeters installed at the toeslope of each watershed. Preliminary results from 2024–2025 indicate that the BAU system produced significantly greater runoff volumes, soil loss, and nutrient export compared to both SHMS and MiG systems. Rainfall simulation experiments illustrate these differences, with mean soil loss of 305 lb acre^{-1} in the BAU system compared with 32 lb acre^{-1} in SHMS and 13 lb acre^{-1} in MiG. An exception to this trend was soluble reactive phosphorus, which was elevated in the SHMS and comparable to BAU losses, suggesting that surface-applied manure products may increase dissolved phosphorus transport risk. These preliminary results demonstrate how soil health management and perennial grazing systems can improve the environmental performance and climate resilience of dairy production systems while maintaining forage productivity. The presentation will highlight preliminary edge-of-field runoff, rainfall simulation, and vadose-zone leachate results collected during 2024 and 2025. This research is supported by the USDA Foundation for Food and Agriculture Research, Dairy Management Inc., and the Wisconsin Dairy Innovation Hub.

Differential sensitivity of milk protein and milk yield to heat stress load in a robotic pasture-based dairy system

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The objective of this retrospective investigation study was to quantify the longitudinal impacts of the temperature-humidity index (THI) on milk yield (MY) and milk composition of Holstein-Friesian lactating cows within a robotic pasture-based dairy system. An 8-year dataset (2016–2023) comprising 2,594 herd records from University of Melbourne's Dookie robotic dairy farm, was matched with the meteorological data collected from the nearest weather station to the farm. Statistical analyses were conducted using PROC MIXED (SAS 9.4) for mean comparisons and segmented (broken-line) regression (PROC NLIN) to identify the critical THI breakpoints associated with MY and milk protein concentration. Adjusted THI, incorporating wind speed and solar radiation, was applied in the segmented regression analysis to better represent heat load under the high solar exposure conditions, typical of Australian pasture-based dairy systems. Pearson correlation analysis assessed the relationship between the same-day (d 0) and lagged THI (1–3 d) with MY and milk protein content. An increase in THI was associated with reduced average herd MY and milk protein concentration ($P < 0.05$). When stratified by a 30-kg threshold based on average daily herd milk production, stronger negative correlations with THI were observed above the threshold (≥30 kg/d), peaking at a 3-day lag ($r = -0.406$; $P < 0.001$), whereas associations were weaker and inconsistent below this threshold (i.e., < 30 kg/d). For milk protein content, there was an overall significant negative association between THI and milk protein content ($r = -0.495$ to -0.524; $P < 0.001$), with the strongest correlations observed at 3-day lag and the 3-day rolling average. The inverse relationship was more pronounced when average daily herd MY was below the lower threshold (either <25 or <30 kg/d) compared with upper threshold (≥25 or ≥30 kg/d), indicating greater protein sensitivity to heat load than MY at lower production levels. Segmented regression analysis based on adjusted THI identified significant breakpoints for milk protein concentration at approximately 41.7 for 3-day average THI to 43.5 for same-day (d 0) THI, beyond these determined THI breakpoints, milk protein declined significantly ($P < 0.001$). The greatest reduction was determined to occur for the 3-day average THI, indicating a cumulative heat load effect on milk protein concentration. In contrast, breakpoints for MY were estimated at approximately 55.2 to 57.1 THI units, but post-breakpoint slopes were not significant, suggesting that there is no evident evidence in MY decline beyond the determined breakpoints. In conclusion, in pasture-based, robotic dairy system, cumulative heat stress impaired milk protein concentration more consistently than milk production. Although MY showed stronger short-term associations with THI at a higher average daily herd MY, milk protein was more sensitive under lower production level and responded at lower adjusted THI thresholds. These results likely suggest that monitoring milk protein may enable earlier detection of heat stress and support proactive heat mitigation strategies in pasture-based robotic dairies.

Summer-smart strategies for livestock production in temperate zones: supplementation of grazing cattle

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In the context of climate change and increased frequency of droughts, summer supplementation of grazing cattle may improve productivity and resilience of pastoral systems. The productive response to supplementation must be studied along with physiological indicators related to heat stress as feeding strategies may have consequences in heat production. The aim of this study was to evaluate the productive response of providing energy-protein supplements to growing steers grazing native grasslands in summer and to evaluate the effects of the supplementation on respiration rate (RR) and body temperature (BT) in a temperate region. Three independent studies were conducted over three summers (2020-2023) in the 'Palo a Pique' long term research platform located in eastern Uruguay (33.14° S, 54.15° W). Each experiment consisted of two treatments: control without supplementation (CONT) and supplementation (SUPPL) replicated in three 4-ha blocks in a completely randomized block design. Stocking rate varied between years (1.5 – 2.5 animals/ha) and steers had ad libitum access to artificial shade (4 m2/animal) and water on a continuous stocking system. SUPPL steers were group-fed in the morning three days per week with an energy-protein ration (16% CP, 13% ADF, 25% NDF, 78% TDN) at a level of 1.98% body weight (BW) on days of feeding. RR was measured by visual observation at 08:00 and 14:00 h, and BT was recorded continuously at 1 h intervals using an automatic sensor implanted subcutaneously. Data was analyzed using a mixed model with treatment and block as fixed effects and year as the random effect. According to the temperature-humidity index (THI), cattle were exposed to alert (74 < THI < 79), danger (79 < THI < 84) and emergency (THI ≥ 84) heat stress conditions 23%, 8%, and 1% of the experimental period, respectively. Sward height at the beginning of the grazing season was similar ($P > 0.05$) between treatments (CONT = 4.6 cm, SUPPPL = 4.2 cm, SEM = 0.2), but CONT paddocks had lower final sward height than SUPPL paddocks ($P < 0.05$) at the end of grazing (4.8 and 5.5 cm, respectively). The pasture had a medium-low content of protein (6.1 – 9.6% CP) and high content of fiber (60 – 64% NDF), which is typical of native grasslands dominated by C4 grasses in summer. Supplemented steers were heavier ($P < 0.05$) at the end of summer than CONT steers (347 and 313 kg/a; SEM = 5) due to ADG significantly greater ($P < 0.05$) than CONT animals (0.90 and 0.45 kg/a/d; SEM = 0.07). The use efficiency of the supplement was 6.8 kg of DM supplement consumed per kg of observed added gain (min. 10.1 and max. 5.1). RPM of SUPPL and CONT animals was similar at 08:00 h (43 ± 1 rpm; $P > 0.05$), but SUPPL animals showed higher ($P < 0.05$) RPM than CONT animals at 14:00 h (63 ± 1 rpm and 57 ± 2 rpm, respectively). Although the daily mean BT was similar between treatments ($P > 0.05$), SUPPL steers had higher BT ($P < 0.05$) than CONT steers between 08:00 and 12:00 h (38.8 ± 0.1 °C and 38.3 ± 0.1 °C, respectively), with the greatest difference after delivering the supplement (09:00 h) averaging 39.1 ± 0.1°C (SUPPL) and 38.1 ± 0.1°C (CONT). Summer supplementation improved the performance of grazing steers. Although it temporarily increased BT (morning) and RR (afternoon), daily average RR and BT were similar for both treatments. This 3-year evaluation provides novel experimental evidence that summer supplementation improves animal performance without significantly increasing the risk of heat stress in grazing animals in a temperate zone.

Fifty years of progress for beef cattle production in the United States

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Many changes in feed production and the maintenance and genetics of cattle have occurred over the past 50 years which affect the efficiency, productivity and environmental impacts of beef cattle production. Trends in feed and animal productivity were determined through regression of historical annual data with time. The Integrated Farm System Model, following life cycle assessment procedures, was used to assess the impact of these changes by modeling representative cattle operations throughout the US in 1972 and 2022. Improvements in yields of feed crops, animal maintenance and genetics, machinery efficiency, and the production of resources such as fuel, electricity, and fertilizer have led to many reductions in the life cycle environmental impacts of beef cattle production. Fewer but larger cattle harvested in 2022 produced 23% more beef carcass weight than in 1972. Nearly the same total feed was consumed to produce beef in 2022 as used in 1972, but about 9% less forage and 43% more grain and other concentrate feeds were fed. Increases in crop yields, particularly corn yields, led to a 12% reduction in cropland and 11% reduction in total land use over the 50-yr period. Other predicted improvements included a 25% reduction in blue water use, 23% reduction in fossil energy use, and 6% reduction in methane emissions. There was little change in total reactive N losses related to beef cattle production with a 34% increase in ammonia emissions offset by decreases in N denitrification, leaching and runoff losses. Considering the increase in animal weight produced, reductions per unit of carcass weight included 21% less feed, 29% less land, 41% less blue water, 39% less fossil energy, 26% less methane, and 19% less reactive N loss. A sensitivity analysis showed that these results were not influenced much by changes in our assumptions on past animal and pasture management. These improvements reflect the continuing trend of the U.S. beef industry to increase productivity and efficiency for a more sustainable product to meet the growing demand for beef. Key words: beef cattle, Integrated Farm System Model, life cycle assessment

Climate change and thermal stress in cattle: Global projections with high temporal resolution

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Cattle farming is a cornerstone of global agriculture, providing essential protein, dairy products, and livelihoods for billions of people. However, the industry is increasingly vulnerable to climate change, particularly thermal stress—the physiological strain caused by the combination of high temperature and humidity, which constitutes one of the most significant challenges to livestock welfare and economic viability. While previous studies have mapped general warming trends and their expected effects in the cattle industry, they lacked the high-resolution temporal data necessary to capture ”micro-dynamics” of heat stress, such as the duration of night-time recovery periods, and the expected frequency and duration of heatwaves. To quantify future risk, we based our analysis in the Temperature-Humidity Index (THI), a widely recognized metric for assessing the combined impact of heat and moisture on livestock physiology. By building on recent work that used a machine-learning model to convert daily climate projections into hourly THI values, our study projects the duration and intensity of thermal stress periods, as well as the frequency and duration of thermal stress waves, under different greenhouse gas emission scenarios, extending to the end of the 21st century. Our results reveal a progressive increase in both the intensity and duration of thermal stress across the globe, with severity largely dependent on future greenhouse gas emission trajectories. While currently most cattle throughout the world can experience sporadic periods of mild heat stress, conditions creating severe heat stress (where mortality risks rise) are quite rare. However, our data projects that under a ”business-as-usual” emissions scenario, the average duration of severe heat stress periods could increase up to 12-fold by the late century; and even under a moderate emissions scenario, the global average duration of severe heat stress is likely to increase nearly 6-fold during the same period. Our results also project a significant lengthening of the duration of Thermal Stress Waves. By late century, several regions will move from experiencing relatively short heat spikes to enduring prolonged and intense thermal stress waves, which are particularly concerning because they are strongly linked to increased mortality in livestock. When future thermal stress projections are combined with global cattle distribution, clear risk hotspots appear across several regions, including South and Central America, East and Central Africa, South and Southeast Asia, and parts of Oceania. These regions are expected to see a disproportionate share of negative impacts of heat stress on their cattle farming operations. We conclude that urgent mitigation and adaptation strategies are needed. Physical adaptations, including shaded structures, hydration stations, and optimized barn ventilation, must be prioritized in tropical and sub-tropical regions. The implementation of genetic improvement programs for breeding heat-tolerant animals would create another layer of resilience for the sector and the populations it supports.

Fans, sprinklers, or what else? Understanding Swedish farmers' experiences with cooling strategies

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Following the 2018 heat wave across Europe, Swedish dairy farmers have become increasingly aware of heat stress in cows, and fans on farms are becoming popular. Research from countries with warmer climates shows that fans in combination with evaporative cooling are the most effective mitigation strategies. Yet it is unknown whether such strategies are applicable in a Nordic climate like that in Sweden. Neither is it known what farmers' current experiences or views are on mitigation strategies for heat stress. Our aim was to better understand which short- or long-term cooling methods for cows were currently used by Swedish dairy farmers and the challenges they encountered. Based on a reflective thematic analysis of 13 interviews with Swedish dairy farmers, several patterns were identified regarding on-farm heat-stress mitigation. Fans were consistently described as the primary investment to alleviate heat stress. The majority reported installing them to complement the natural/passive ventilation in the barn or milking station. However, farmers who had installed fans described variable benefits. This suggests that fans might not be a complete solution, as animals were still reported to show signs of heat stress (e.g., reduced milk production, reduced activity, or panting). Reports on how farmers selected the type, number, or placement of fans reveal a lack of context-specific strategy or guidance in Sweden. Moreover, fan installations were often used not for actual cooling but to maintain or improve airflow in the barn on hot days, with the belief that this is key to preventing cows from going into heat stress. On the other hand, water-based cooling strategies were viewed cautiously. While some farmers experimented with improvised solutions, such as showering cows or constructing homemade sprinklers, concerns were often raised about increased barn humidity, increased manure volume, hygiene problems, and disease transmission, and in some regions, it was deemed infeasible due to water scarcity. Genetic selection was also discussed with farmers. This was seen as a potential long-term resilience strategy. Here, farmers perceived limited individual influence and often framed heat tolerance as already indirectly addressed through breeding for general robustness and disease resistance. Overall, heat stress mitigation was not described as a top priority due to the relatively short Swedish summer season. Thus, any actions undertaken on the farm were closely linked to alleviating major problems on farm. Heat mitigation strategies in Swedish dairy cows remains a low priority, as the belief is that the losses seen during summer events are offset by gains during the rest of the year. Currently, fans have shown the greatest uptake on farms but have not successfully resolved heat stress in cows. Thus, water-based solutions must be explored in a Swedish context, taking into account both old and new barn constructions to avoid humidity problems and issues with manure handling, which are seen as factors limiting uptake.

Aligning research gaps and innovation demands for ecosystem services in South American pasture-based livestock systems

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Pasture-based livestock systems dominate beef production across the Southern Agricultural Council (CAS) region of South America and play a key role in food security, rural livelihoods, and ecosystem services. However, increasing societal expectations regarding climate change mitigation, biodiversity conservation, water stewardship, and animal welfare require stronger alignment between scientific research agendas and technological innovation priorities. This study aimed to identify and prioritize scientific and technological gaps related to ecosystem services in pasture-based livestock systems in the CAS region. A structured consultation was conducted within the Sustainable Livestock Platform promoted by the Inter-American Institute for Cooperation on Agriculture (IICA) and the Inter-American Development Bank (IDB). A web-based survey, developed from a prior scientific review, evaluated four strategic domains: carbon balance, water balance, biodiversity, and animal welfare. Between seven and ten specific topics were assessed within each domain. A total of 106 regional experts from Argentina, Uruguay, Brazil, Paraguay, Chile, and Bolivia participated in the consultation, with 95 complete responses. Participants evaluated both knowledge gaps and R+D+i priorities using a five-level scale ranging from low to very high. Results revealed strong convergence between perceived scientific gaps and innovation demands across the four domains. In carbon balance, the highest priorities were systemic research on sustainable livestock systems, improved carbon management practices, and the development of robust greenhouse gas inventories. For water balance, climate change adaptation strategies and watershed-level approaches emerged as key priorities. Biodiversity results highlighted the need for greater understanding of livestock impacts on biodiversity and the establishment of long-term monitoring frameworks. In the animal welfare domain, stakeholder participation, training processes, and the development of practical assessment and monitoring indicators were identified as the main priorities. These findings highlight the need for integrated, long-term research programs addressing ecosystem services in livestock systems and strengthening regional research networks. Improved alignment between science, innovation, and stakeholder demands will be essential to enhance the environmental performance, social legitimacy, and global competitiveness of South American pasture-based livestock systems.

Effect of reproductive status on goat methane emissions

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Goat production is the fastest-growing ruminant production globally and is vital for food security in tropical and subtropical regions. Reducing enteric methane is crucial to enhancing sustainability and productivity. While most studies have focused on nutritional effects, physiological factors such as reproductive status change nutrient demand, dry matter intake (DMI), and rumen function, potentially influencing methanogenesis. To our knowledge, the longitudinal effects of reproductive status on rumen function and methane emissions in goats remain unexamined. We evaluated the effects of reproductive status (dry, pregnant twin-bearing, and early lactation) on goat methane emissions. Forty two-year-old Boer × Rangeland does (n=20 dry; n=20 pregnant) were studied from week -4 prepartum to week +4 postpartum. Animals were housed individually and fed a maintenance diet (lucerne hay, barley hay, and commercial lactating pellets) formulated using the Small Ruminant Nutrition System. Methane emissions were measured using the sulphur hexafluoride (SF6) technique. Data were analysed using a mixed model in SAS (v9.4) with Kenward-Roger adjustment. Post-hoc pairwise comparisons were performed with LSMEANS/PDIF. Statistical significance was declared at $P \leq 0.05$; data normality was assessed with the Shapiro-Wilk test. Reproductive status markedly influenced intake patterns. DMI increased from 0.60 ± 0.08 in week -4 prepartum to 1.07 ± 0.05 kg/d at kidding ($P < 0.001$). Following parturition, DMI increased sharply to 1.35 ± 0.05 in week +1 ($P < 0.001$) and peaked at 1.75 ± 0.06 kg/d by week 4 of lactation ($P < 0.001$). In contrast, DMI in dry does remained stable throughout the experimental period (av. 0.51± 0.04 kg/d; $P > 0.1$). Body weight remained stable in dry and lactating does, while pregnant does showed increases consistent with advancing gestation, which together with weekly condition scoring suggest confirmation of a maintenance-level feeding strategy. Overall, pregnant goats had higher DMI than dry goats (0.62 vs. 0.43 kg/d, respectively; P=0.03). Despite this, both pregnant and dry does emitted comparable amounts of methane (26.51 and 27.06 g/d, respectively; P=0.85), resulting in pregnant does having a lower methane yield than their dry counterparts (47.26 vs 65.91 g/kg DMI, respectively; $P<0.001$). During the first month post-kidding, lactating does had higher DMI than dry animals (1.76 and 0.59 kg, respectively; $P < 0.001$). Similarly, lactating does had higher daily methane emissions compared to dry does (81.66 and 35.57 g/d; $P < 0.001$), yet methane yield in lactating animals was lower than their dry counterparts (47.22 and 60.75 g/kg DMI, respectively; $P<0.001$). Diets met maintenance and reproductive requirements, with pregnant and lactating does receiving higher quality, lower NDF rations, partly explaining lower methane emissions than dry animals. Methane production was primarily driven by DMI. Pregnant does, despite ~45% higher DMI, produced similar methane (g/d) to dry does, likely due to reduced rumen capacity and retention time, and late gestation hormone-driven alterations in rumen metabolism. Lactating does emitted more methane (g/d) due to higher DMI, but had lower methane yields, possibly reflecting faster passage rate and reduced rumen retention time. Overall, pregnant and lactating goats emitted less methane per unit of DMI suggesting lower methane intensity and improved efficiency.

Needs and Capacity building of advisors to support transitions towards climate-smart farming systems

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Faced with the complexity of global challenges such as climate change, market dynamics and environmental sustainability, EU farmers are increasingly reliant on farm advisers to navigate political, technological, social and environmental imperatives (see for example, Ingram et al., 2022). Boosting the capacity of the EU agricultural advisory community to advance climate smart farming is therefore an urgent task. Recognising this urgency, the EU Horizon programme has funded Climate Smart Advisors (CSA) project, a pan-European multi-actor network covering 27 countries started in 2023. Its aim is to boost the EU agricultural advisory community in the field of climate smart farming by strengthening the advisors' capacity and role in the transition towards Climate-Smart (CS) Farming. Within CSA, a range of complementary activities are designed to strengthen the CS advisory capacity of the EU advisory community, including the following: (1) An EU-wide network of 260 advisory Communities of Practice (CoP) to support the development of 1500 advisors to form the core of CS knowledge exchange; (2) Expert training for 140 advisors on selected topics, relevant to their context and for facilitating a CoP; (3) Knowledge exchange in 12 thematic areas across the CoPs; (4) A knowledge repository for advisors with CS tools, practices, and approaches (5) Monitoring, evaluation, and formative learning activities to capitalize on lessons being learned within and outside the project. With this communication, we want to discuss the needs and capacities agricultural advisors require to support farmers in implementing sustainable and climate smart farming. It builds on the first two year of monitoring, evaluation, and learning within the Climate Smart Advisors (CSA) network. Our analysis draws from multiple sources, including a training needs assessment of 80 advisors, a literature review, advisors' focus groups, insights from the first-year evaluation of the CoPs and the learning questions identified by advisors in the CoPs. We first analyse farmers' needs and then dig-in into the advisors' needs to support farmers. The needs of advisors are related to (1) deeper and more nuanced knowledge about climate science, its impacts on agriculture, and the practical measures that can mitigate these effects. This includes understanding the specific challenges faced by different farming systems.(2) Second, advisors call for better tools and resources to aid their work. This includes digital platforms for data visualization, monitoring tools to assess climate impacts, and repositories of case studies and best practices that can be shared with farmers. Many CoPs of the network are working on building together such tools and ressources. (3) Finally, advisors express a need for guidance on engagement strategies, particularly when dealing with resistant or skeptical farmers. They seek methods for navigating difficult conversations, fostering collaboration, and maintaining momentum in group settings. (4) Additionally, advisors emphasize the importance of networking and collaboration with their peers, researchers, farmers and policymakers to stay informed, share experiences, and co-develop solutions that are both innovative and practical.

Integrating thermal load and metabolic indicators to support climate smart management decisions in dairy herds

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More frequent and intense heat events challenge the resilience of dairy systems and threaten productivity and health of the animals , making climate-smart management essential for securing global protein supply. Conventional heat stress indicators such as the temperature–humidity index (THI) describe the environment but overlook individual metabolic responses that determine actual performance and welfare outcomes. Holistic indicators that combine barn climate, animal-level physiology and routine farm data could help farmers adapt to warming conditions while reducing avoidable losses and associated emissions. This study aimed to (i) quantify relationships between weather station (WS-mean3THI), barn- (Barn-mean3THI) and cow-level THI (Cow-mean3THI), housing type and key production, metabolic and energy-balance (EB-NEL) traits; (ii) evaluate how dry matter intake indicator (DMI) relates to energy-corrected milk (ECM); and (iii) assess whether combining THI with metabolic information improves interpretation of heat stress effects compared with THI alone, thereby supporting climate-smart management decisions. Data were collected during summer in commercial dairy herds of major dairy breeds housed in cold open barns or warm freestall barns. Environmental records were used to compute 3-day moving average THI. Milk yield (MY), ECM, milk composition (fat, protein, lactose, urea, somatic cell count), and detailed milk fatty acid (FA) profiles were combined with milk and blood biomarkers (e.g. BHB, NEFA, acetone), derived energy-efficiency traits (EE-NEL), feed efficiency (FE-ECM) and EB NEL and DMI. Correlation analyses explored associations among climate, production, metabolic and FA traits. General linear models (GLM) analysed (i) Cow-mean3THI increased with WS-THI class and was consistently higher in warm than in cold housing, indicating that warm barns increase effective heat loadunder comparable outside conditions. WS-mean3THI showed large, clearly separated differences between “cool”, “moderate” and “hot” classes, whereas the additional housing effect on barn THI was small. Higher THI was associated with less favorable EB-NEL, elevated ketone body concentrations and shifts in milk FA towards higher proportions of long-chain and preformed FA, consistent with increased body fat mobilization. In the ECM model, WS-mean3THI and housing type had significant effects, while the effect of Cow-mean3THI became negligible after including WS-mean3THI and DMI. DMI was strongly associated with ECM and EB-NEL traits, indicating that cows with lower DMI status produced less ECM under similar climatic conditions. The results confirm that housing design influences individual heat load and that WS-mean3THI provides a simple, operational indicator of transitions from comfortable to stressful conditions. However, THI alone does not adequately explain performance and health outcomes; combining THI with DMI and EB-NEL indicators substantially improves interpretation of heat stress impacts. Integrated milk spectral data indicators derived from on-farm sensor, milk-recording and laboratory data can act as early-warning tools for heat-related metabolic stress and ECM losses. In the context of climate-smart livestock systems, these holistic, data-driven indicators can support adaptive management by identifying high-risk periods, prioritizing vulnerable animals and guiding targeted interventions, thereby contributing to more resilient and resource-efficient dairy production.

Effects of dietary supplementation with a novel marine algae–derived prebiotic feed additive (MAFA) on growth performance, gaseous emissions and manure characteristics from a commercial finishing pig facility

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Ammonia and greenhouse gas (GHG) emissions from agriculture pose significant environmental and health concerns. Nutritional strategies, including the use of feed additives, have been proposed to enhance animal performance while reducing gaseous emissions from livestock facilities. This study evaluated the effects of dietary supplementation with a marine algae–derived prebiotic feed additive (MAFA) on growth performance, gaseous emissions and manure characteristics of finishing pigs housed in a fully slatted, mechanically ventilated commercial facility. Two experiments were conducted using pigs of uniform initial body weights. The studies utilized two separate and identical fully slatted finishing pig rooms, measuring 12 m × 9 m, with manure tanks depth of 1.4 m. Each room is equipped with a negative-pressure ventilation system. Pigs were moved into the finisher room 2 weeks before the start of the trial, were arranged into pens of 10-11 pigs of similar weight and were fed the control diet. At the same time, slurry pits were emptied to similar levels across all rooms. A total of 164 pigs were used in the first experiment and 185 pigs in the second. Diets were supplemented with MAFA at inclusion levels of 0.5 or 1.0 kg/tonne and compared with unsupplemented controls. The first experiment (0.5 kg/tonne) was conducted from March to May 2025, while the second experiment (1.0 kg/tonne) was conducted from June to September 2025. Ammonia and greenhouse gas emissions were monitored for 56 days (0.5 kg/tonne) and 53 days (1.0 kg/tonne) using a Gasera photoacoustic gas analyser. Gaseous monitoring followed the VERA test protocol and emission calculations accounted for outliers, mortality, pig numbers and incomplete monitoring days. Productive performance data were analysed using a general linear model, while model assumptions and homogeneity of variance were verified prior to applying appropriate parametric or non-parametric tests. Results found that MAFA supplementation did not affect productive performance ($p > 0.05$) at either inclusion level. Ammonia emissions were also unaffected ($p > 0.05$). Greenhouse gas responses were gas-specific and dose-dependent. Carbon dioxide emissions differed at 0.5 kg/tonne ($p = 0.030$), whereas no differences were observed at 1.0 kg/tonne ($p > 0.05$). Methane emissions were unchanged at 0.5 kg/tonne but decreased by 41.8% at 1.0 kg/tonne ($p < 0.001$). In contrast, nitrous oxide emissions increased at both inclusion levels ($p < 0.001$). Manure analysis showed a modest reduction over time ($p < 0.05$) in fresh manure pH with MAFA supplementation at 1.0kg/tonne, along with changes in dry matter and nutrient composition, including ammonium-N, phosphorus, and potassium concentrations. However, these changes were insufficient to reduce ammonia emissions at the housing level. Overall, supplementation with a marine algae–derived prebiotic feed additive under commercial finishing conditions did not improve productive performance or reduce ammonia emissions but influenced greenhouse gas dynamics and manure chemistry.

Impact of dietary calcium and phytase on mineral digestibility and skeletal mineral accumulation in growing pigs.

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An optimal dietary calcium (Ca) to phosphorus (P) balance is essential to minimize the use of inorganic phosphates in pig diets. This study evaluated the effects of dietary Ca and microbial phytase (PhytM) supplementation on nutrient digestibility and bone mineralization in growing pigs, with PhytM added by partially replacing monocalcium phosphate and limestone. A total of 240 pigs were allocated to 40 pens. Each pen was assigned to one of five diets: a control Ca diet without PhytM (CCa-PhytM0), CCa + 500 FTU/kg PhytM (CCa-PhytM500), CCa + 2000 FTU/kg PhytM (CCa-PhytM2000), low Ca (LCa-PhytM0), and LCa + 500 FTU/kg PhytM (LCa-PhytM500). All diets were formulated to provide 85% of the P requirements for 25-to 50-kg pigs. Total Ca to digestible P ratios were 2.3 for the CCa diets (CCa-PhytM0, CCa-PhytM500, CCa-PhytM2000) and 1.3 for the LCa diets (LCa-PhytM0, LCa-PhytM500). Body composition was assessed by using dual-energy X-ray (DXA) absorptiometry scans at the start and end of the experiment and Ca and P deposition were calculated from these values. Statistical analyses were performed using a one-way ANOVA. The first set of contrasts tested the effects of dietary Ca, PhytM, and their interaction using the LCa-PhytM0, LCa-PhytM500, CCa-PhytM0, and CCa-PhytM500 diets. A second set compared CCa-PhytM2000 with CCa-PhytM500. Relationships between femur bone mineral content (BMC) and whole-body BMC, as well as metacarpal BMC and whole-body BMC were evaluated using linear regression models. Pigs fed PhytM500 diet improved apparent total tract digestibility (ATTD) of P ($P = 0.003$) and Ca ($P = 0.002$) but reduced daily deposition of bone ($P = 0.004$), Ca ($P = 0.004$), and P ($P = 0.03$) compared with PhytM0. LCa diets decreased daily bone ($P < 0.001$), Ca ($P < 0.001$), and P deposition ($P < 0.001$) without affecting ATTD of P ($P = 0.75$). Pigs fed CCa-PhytM2000 diet had higher daily deposition of bone ($P = 0.02$), Ca ($P = 0.03$) and P ($P = 0.03$) than CCa-PhytM5000. However, the ATTD of P ($P = 0.71$) and Ca ($P = 0.12$) were similar between CCa-PhytM2000 and CCa-PhytM500 pigs. The regression analysis showed that whole-body BMC was strongly associated with femur BMC ($P < 0.001$) but not with metacarpal BMC ($P = 0.65$). Overall, the improvement in P and Ca digestibility with PhytM measured at 21 days was not reflected in Ca and P deposition for 21 days, because digestibility shows status at a specific point in time, whereas body composition reveals a cumulative effect. Accordingly, the current results showed that apparent digestibility does not consistently capture the true impact of PhytM on bone mineral accretion. These findings underline the need to combine digestibility measurements with physiological indicators or bone mineral responses to obtain a reliable evaluation. Additionally, this study emphasises the need to use a bone that is truly responsive to dietary strategy.

Nutritional Strategies to Enhance Phosphorus Utilization Efficiency in Pigs

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Optimizing phosphorus (P) nutrition in growing pigs is essential for improving the sustainability of the swine industry and the food system. Plant-derived phosphorus is the primary source of P in pig diets, with approximately 70% present as phytic P (PP). However, this form is largely indigestible for growing. Consequently, supplementation with inorganic phosphate sources is typically required to meet the animals' phosphorus requirements. The main feeding strategy to improve PP utilization is the inclusion of phytase in pig diets. With the high efficacy new generation of phytases, it is now possible to meet P requirements without the addition of inorganic phosphate sources, except during the early growth phase. Animals possess adaptive mechanisms to cope with certain mineral deficiencies, particularly P and calcium (Ca) by enhancing digestive utilization and increasing the efficiency of utilization of the limiting nutrient. This study aimed to evaluate the effects of different feeding strategies on growth performance, bone mineral content (BMC), and the efficiency of total P and digestible P (dP) utilization. To achieve this objective, two experiments were conducted in 2025 using pigs of the same genetic line and control diet. Animals had ad libitum access to water and feed meeting nutritional requirements of growing pigs (NRC, 2012), except for P and Ca. In experiment 1, 192 pigs (31.2 kg) were randomly allocated to 24 pens (6 pigs/pen) and received four dietary treatments across three phases (21, 28, and 28 d) until 130 kg: (1) NRC (85% maximal bone mineralization), (2) Control (Lautrou et al. (2020); 100% dP and Ca), (3) High (150% of treatment 2), and (4) Phytase (1,000 FTU; equivalent to treatment 2 in phase 1 and treatment 3 in phases 2–3 without phosphate). In experiment 2, 114 pigs (38.7 kg) were housed in pens of 12 and received either Control or Depletion (60% dP and Ca of treatment 2) across two phases (28d depletion, 56d repletion (fed with control feed)) until 130 kg. Eight pigs per treatment were scanned by Dual-X-ray (DXA) to determine BMC, which was converted to body P and Ca allowing to calculate the efficiency as P retained/P total or dP intake. The different feeding strategies did not affect average daily feed intake, average daily gain, or feed conversion ratio. However, daily BMC gain was higher in High (+25%) and Phytase (+26%) compared to Control ($P < 0.001$). This indicate that although body weight was not affected, BMC increased with higher dietary P and Ca levels. Total P utilization efficiency was influenced by feeding strategy ($P = 0.01$). The Phytase (55%) treatment showed better P utilization than the High (46%; $P < 0.001$), Depletion (49%; $P = 0.034$), and NRC (47%, $P < 0.001$), and tended to be higher than the Control diet (51%; $P = 0.07$). Feeding strategy also affected dP utilization efficiency ($P < 0.001$). The Depletion (90%) treatment showed greater efficiency than the Control (78%; $P < 0.001$), NRC (73%; $P < 0.001$), Phytase (68%; $P < 0.001$), and High (62%; $P < 0.001$), with significant differences observed among treatments. This indicate that depletion allow to retain more dP probably due to homeostasis mechanisms. Overall, this study demonstrates that growing pigs can be fed diets supplemented with phytase without the need for inorganic phosphate, achieving the highest total P utilization and, the lowest P excretion. The results also indicate that when dP is limited, pigs can partially compensate through adaptive regulatory mechanisms that enhance P utilization. Incorporating P utilization efficiency into feed formulation strategies could therefore offer both economic benefits and environmental advantages.

Valorization of native trace minerals in alternative feed ingredients for sustainable pig production

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Phytase supplementation is widely used in pig nutrition to improve the availability of phosphorus (P) and trace minerals bound to phytate in plant-based feeds. While effective in grain-rich diets, its efficacy may differ in diets with inherently low phytate levels, such as those containing processed animal proteins (PAP). In such systems, relying solely on phytase may not provide sufficient P and trace minerals to meet piglet requirements. This study evaluated the effect of withdrawing inorganic phosphorus and trace mineral supplementation on mineral bioavailability and performance in PAP-based nursery diets. A total of 192 piglets (Pietrain × TN70), weaned at 21 days with an average body weight of 6.8 kg, were assigned to six dietary treatments with four pens of eight pigs per treatment. Diets differed in protein source (soybean meal, SBM, or PAP from black soldier fly meal) and in the inclusion of phytase and mineral supplementation (trace minerals + monocalcium phosphate) during the 4-week of trial. Treatments included SBM diets with (SBM+) or without mineral supplementation (SBM−), PAP diets with (PAP+) or without mineral supplementation (PAP−), and two PAP-based negative controls formulated without phytase (NC) or with mineral supplementation only (NC+minerals). Diets were formulated to be isoenergetic and isonitrogenous and produced by ILVO. Growth performance, fecal parameters, serum mineral concentrations, bone mineralization, and mineral retention were measured. Mineral supplementation significantly improved final body weight, average daily gain, feed intake, and feed efficiency ($P < 0.05$). Pigs fed PAP-based diets performed worse than those fed SBM-based diets, suggesting that assumed nutrient values may have overestimated the actual nutrient contribution of PAP ingredients. Phytase supplementation did not significantly affect growth performance. Within PAP diets, mineral supplementation significantly improved performance, whereas phytase alone did not compensate for the removal of minerals. Serum mineral concentrations were largely unaffected, although higher serum P levels were observed with mineral and phytase supplementation at the end of the trial. Phytase supplementation significantly increased bone crude ash, Zn, P, and Mg concentrations and improved Zn retention. However, positive effects of phytase on Zn retention were only observed when minerals were not supplemented. Overall, removing inorganic phosphorus and trace minerals from piglet diets negatively affected growth performance, mineral status, and mineral retention. While alternative protein sources such as PAP contribute to more sustainable pig production, the results highlight the importance of careful mineral nutrition strategies to avoid deficiencies when formulating low-phytate diets.

Terrestrial ecotoxicity and phosphorus excretion are reduced by increasing phytase and reducing nutrient concentrations in growing pig diets

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Environmental impacts must be considered in pig production, and dietary phytase may be an alternative to mitigate some of them. In this context, the aim of this study was to evaluate increasing dietary phytase levels with the respective reductions in phosphorus (P), calcium (Ca) and standardized ileal digestible (SID) amino acid concentrations on the environmental impacts of growing pig production, assessed by life cycle assessment (LCA), as well as on P and nitrogen (N) excretion. Two experiments were conducted. In Experiment 1, Forty crossbred barrows (30.32 ± 0.88 kg initial body weight) were allotted in a randomized block design with four treatments and ten replicates to determine growth performance until 50 kg of body weight. In Experiment 2, twenty-four crossbred barrows (36.55 ± 2.16 kg initial body weight) were housed in metabolic cages and allotted in a randomized block design with four treatments, six replicates, and one animal per experimental unit to determine N and P balances in growing pigs. In both experiments, treatments consisted of diets containing four phytase levels (0, 250, 500, and 1000 FTU) with the respective reductions in P (0.357, 0.297, 0.237, and 0.117%), Ca (0.722, 0.662, 0.602, and 0.428%), SID lysine (0.007% per 250 FTU), SID methionine (0.004% per 250 FTU), SID threonine (0.004% per 250 FTU), SID valine (0.002% per 250 FTU), and SID tryptophan (0.001% per 250 FTU). Based on data obtained from Experiments 1 and 2, environmental impacts were calculated per kg of body weight gain using LCA, including global warming potential (GWP), acidification potential (AP), eutrophication potential (EP), cumulative energy demand (CED), terrestrial ecotoxicity (TE), and land occupation (LO). Data on N and P balance, as well as LCA-derived environmental impacts, were subjected to regression analysis when a significant effect was detected by ANOVA. Nitrogen excretion was not affected ($P > 0.05$) by dietary phytase inclusion. However, P excretions in feces ($P < 0.0001$) and urine ($P < 0.0001$) decreased linearly as phytase levels increased, resulting in a linear reduction in total P excretion ($P < 0.0001$). These results may reflect the linear decrease in P intake ($P = 0.0075$). However, the retained-to-intake P ratio improved ($P = 0.0007$) as phytase levels increased, demonstrating the effectiveness of phytase as a nutritional strategy. The LCA showed no differences for GWP ($P = 0.3501$), AP ($P = 0.2552$), EP ($P = 0.8556$), CED ($P = 0.8300$), or LO ($P = 0.5453$). However, increasing dietary phytase levels resulted in a linear reduction in TE ($P = 0.0001$), from 9.44 to 7.95 g 1,4-DBC-eq, indicating reduced adverse effects on soil ecosystems, microorganisms, plants, and fauna. In conclusion, increasing dietary phytase levels with the respective reductions in P, Ca, and SID amino acid concentrations reduced terrestrial ecotoxicity and phosphorus excretion, thereby mitigating some environmental impacts of growing pig production.

Comparative Analysis of Three Approaches for Estimating Phosphorus Retention in Growing Pigs: Implications for Phosphorus Excretion Estimates

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Phosphorus (P) is essential for crops and animals, and its use must be optimized to support sustainable agriculture as it's a non-renewable resource. Dietary strategies have been developed to reduce P excretion in animal production, and large variations in both intake and excretion can occur without affecting growth performance. By accurately predicting P retention and excretion in pigs, it may be possible to tailor dietary strategies to match crop P requirements. Accurate estimation of P excretion in pig production depends especially on reliable predictions of body P retention. However, retention estimates change based on the method used to determine body P content. This study aimed to compare three approaches for estimating P retention in growing pigs and to assess their capacity to predict P excretion from known P intake. The experiment was conducted with 96 growing pigs allocated to a 2 × 2 factorial design crossing two levels of dietary nitrogen and phosphorus, resulting in four treatments. The experimental unit was the chamber (four pigs per chamber; n = 12 x 2 batches). Body composition was assessed using dual-energy X-ray absorptiometry (DXA). Two pigs per chamber were scanned and average lean and fat values were used to represent each chamber. Three approaches were compared at chamber level: (1) DXA, a DXA-based approach to estimate body P content and retention over the experimental period; (2) BodyComp, an approach based on tissue composition in Ca and P assuming a fixed protein and lipid body composition combined with prediction of Ca and P into bone and tissues, (3) Gain, an approach using a fixed coefficient of P retention per kg of body weight gain. For each method, P excretion was calculated as P intake minus P retention and compared with measured P in slurry. The capacity of prediction was assessed using R^2 and Mean Square Prediction Error (MSPE, %) which was decomposed into error of central tendency (ECT), error of regression (ER) and error of disturbance (ED). All methods showed high $R^2 = 0.83–0.90$ and low MSPE (below 5%). The DXA approach resulted in substantially lower MSPE (1.31%) compared with BodyComp (4.26%) and Gain (3.73%). The DXA error is mainly ED (46%) followed by ER (38%) suggesting a tendency to slightly underestimate P retention. The BodyComp approach error of prediction was mainly ECT (65%), indicating a systematic underestimation of retention. Finally, the Gain method showed 44% ECT and 31% ER. Despite not using DXA information, the BodyComp model achieved a same coefficient of determination (0.90) comparable to the DXA-based approach with an ECT that can be easily corrected. These results suggest that refining non-DXA approaches could improve predictive performance and support reliable large-scale estimations of P retention and excretion with growth performance information only. Refining this approach will allow the P supply to pigs to be adjusted to align with that of crops.

Pig gut microbiome functional genes associated with protein metabolism along the gastrointestinal tract

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The pig gut microbiome is involved in nutrient digestion, and its functional capacity changes along the gastrointestinal tract (GIT). Understanding how local microbial communities affect protein utilization is important for improving nitrogen use efficiency (NUE) in pig production. Studies using shotgun metagenomics to investigate the pig gut microbiome along the GIT are still limited. This study aimed to characterize gut microbiota composition and functional gene profiles, with a focus on peptidases, across GIT sections and to assess their association with protein digestion and fermentation in pigs. Forty-eight male pigs received a two-phase diet based on barley, wheat and soybean meal. During the main phase, digestible lysine covered 90% of the requirement to challenge protein utilization. At 21 weeks of age, pigs were euthanized and digesta were collected from stomach, small intestine, caecum and colon. Urinary and faecal nitrogen excretion and blood metabolites were measured and used together with digestible nitrogen intake to estimate NUE by multiple regression. Short- and branched-chain fatty acids and amino acids were quantified in digesta by NMR spectroscopy. Shotgun metagenomic sequencing (Illumina NovaSeq 6000) was performed on DNA extracted from digesta. Taxonomy was assigned with Kraken2. Metagenome-assembled genomes (MAGs) were reconstructed, and peptidase-encoding genes were identified by annotation against the MEROPS database. Gut microbial community structure differed between GIT sections. Bifidobacterium was most abundant in the small intestine, whereas Segatella dominated the large intestine. Lactobacillaceae was the most abundant family in all sections, and genus-level diversity was higher in the large intestine than in the stomach and small intestine. Predicted transcripts per million (TPM) of microbial peptidases were higher in the stomach and small intestine than in the large intestine. In total, 610 MAGs with completeness above 70% and contamination below 10% were recovered. Peptidase gene copy numbers were highest in Pantoea, Escherichia and Ralstonia. Streptococcus alactolyticus contributed strongly to extracellular peptidase S15, with increasing contribution from proximal to distal GIT sections. Volatile fatty acid concentrations increased in caecum and colon and showed a positive correlation with Bifidobacterium abundance. NUE was positively associated with several functional genes, and these associations differed by GIT section. This study describes the spatial organisation of the pig gut microbiome and its potential role in protein metabolism along the gastrointestinal tract.

Transcriptomic signatures of nitrogen use efficiency in liver and muscle of Swiss Large White pigs under protein-restricted feeding

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Improving nitrogen use efficiency (NUE) in pigs is an important strategy to reduce nitrogen emissions from livestock production while maintaining productivity. However, the molecular mechanisms underlying variation in NUE remain poorly understood. In this study, we investigated transcriptional differences between pigs with high and low NUE in two key metabolic tissues, liver and skeletal muscle. Animals were selected from a population of Swiss Large White pigs raised under a protein-restricted diet and phenotyped for NUE based on feed intake and carcass protein deposition measured using dual-energy X-ray absorptiometry. Individuals from both ends of the NUE distribution were targeted for RNA sequencing of liver and longissimus thoracis muscle. Differential expression analysis identified 177 genes in liver and 133 genes in muscle that differed between high- and low-NUE pigs. Functional enrichment analyses revealed that overrepresented and enriched pathways and ontologies in liver were predominantly associated with amino acid metabolism, nitrogen recycling and amino-acid catabolism, including pathways related to serine, glycine and cysteine metabolism. Several genes involved in inflammatory signalling and acute-phase responses were downregulated in high-NUE pigs, suggesting reduced allocation of nitrogen to immune processes. In muscle, pathways and ontologies were linked to nutrient partitioning, mitochondrial metabolism and protein turnover, including genes involved in lipid uptake, translation initiation and muscle growth pathways. Only one gene (SHMT2), involved in mitochondrial one-carbon metabolism, was differentially expressed in both tissues. Together, these results indicate that variation in NUE reflects coordinated, tissue-specific metabolic adaptations involving amino-acid metabolism, immune activity and nutrient partitioning. These findings provide new insights into the biological mechanisms underlying NUE and may help prioritize candidate genes for genomic selection aimed at reducing nitrogen emissions in pig production.

Phenotypes for implementing proxies of mineral utilisation and bone turnover in pig breeding

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Phosphorus is an essential mineral for skeletal development in pigs; however, its utilization efficiency remains a challenge in modern pig production. Phosphorus and calcium homeostasis are regulated through complex physiological mechanisms involving absorption, retention, and excretion processes mediated by multiple sensing and regulating factors, including the parathyroid hormone (PTH), vitamin D, fibroblast growth factor 23 (FGF23), and the calcium-sensing receptor (CASR). Variability in these physiological pathways may result in measurable differences in circulating mineral concentrations and bone turnover markers. Increasing evidence suggests that part of this variability is genetically determined, influencing mineral absorption, bone mineral deposition, and remodeling activity. Therefore, phenotypes derived from serum mineral levels and biochemical markers of bone formation and resorption may serve as biologically meaningful proxies of mineral utilization efficiency. Understanding the genetic architecture underlying these indicators is essential to evaluate their suitability for incorporation into breeding programs aimed at improving skeletal health and nutrient efficiency. A recent Genome-Wide Association Study conducted by our research group identified genetic loci associated with circulating vitamin D metabolites and bone remodeling markers in Landrace pigs, highlighting genomic regions potentially contributing to bone integrity. This study aims to estimate the variability of phenotypic markers related to phosphorus homeostasis and bone integrity, identify candidate genes through genome-wide analysis as well as validate and integrate new phenotypes reflecting phosphorus utilization and bone integrity in breeding programs. In the first phase, phenotypic variability of serum traits and structural and functional bone properties was assessed to establish a representative sample panel of 160 performance-tested animals from seven pig breeds and commercial crossbreds. All tested traits showed a considerable variation. Overall mean serum concentrations of inorganic phosphorus and calcium were 11.10 mg/dL and 10.30 mg/dL, respectively, ranging from 7.90 to 14.60 mg/dL and 6.50 to 13.80 mg/dL. The bone remodeling markers, such as bone-specific alkaline phosphatase and C-terminal telopeptides of type I collagen also showed considerable variability, ranging from 25.06 to 81.17 µg/L and 0.09 and 0.69 ng/mL, respectively. Principal component analysis to select divergent animals for the development of an ultrasound-based bone assessment method showed major contributions from metacarpal bone circumference, inorganic phosphorus, and ß-CTX. The second phase will comprise 1,000 Swiss Large White pigs to integrate phenotypic and genomic datasets to develop selection tools for improved skeletal health and mineral efficiency. These results are expected to enhance phosphorus utilization efficiency and bone stability, thereby improving animal welfare and reducing the environmental impact of pig production. This work has received funding from the Federal Ministry of Research, Technology and Space (BMFTR) in Germany and Innosuisse – Swiss Innovation Agency in Switzerland – Project number 4908.

A mechanistic model to simulate the digestive fate of calcium and its interaction with phytate and phytase in growing pigs.

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Calcium (Ca) plays a central role in growth, skeletal development and bone mineralization in growing pigs, but may be an antagonism to phosphorus (P) utilization. As its digestive fate is influenced by interactions with phytate and exogenous microbial phytase (PhytM), a deeper understanding of the mechanisms underlying Ca interaction with phytate degradation in the gastrointestinal tract could help maximising phytase efficacy. A mechanistic representation of Ca, and P fluxes along the intestine would also improve our understanding of the factors influencing their bioavailability and the extent to which phytase improves it. Modelling provides a framework to integrate existing knowledge and test hypotheses regarding the functioning of biological systems. The model simulates the process of Ca, P and phytic phosphorus (PP) digestion in the gastrointestinal tract of a growing pig. The stomach is considered as a single compartment well mixed in which solubilization of Ca and P forms and hydrolysis of PP by PhytM are represented. These fluxes are driven by transit time and pH, the latter being dynamically represented considering diet pH and acid binding capacity. To represent the interaction among Ca, P and Zn and the tubular structure of the small intestine (SI), SI is represented as a serie of repeated structural units referred to as segments. Each segment consists of 14 state variables representing mineral pools in soluble and insoluble forms. Insolubilization of PP in the SI is the driving force of formation of Ca-PP complexes considering that one mole of PP binds up 3 moles of Ca. Absorption of soluble Ca, P, and Zn from the intestinal lumen to the blood was assumed to occur via both saturable transporters and passive mechanisms. Model was evaluated using the predictions of the apparent digestibility (AD) of Ca, P and PP. Results were evaluated against independent literature data on apparent digestibility (5 studies; 29 dietary treatments for PP, 15 studies; 79 dietary treatments for Ca, and 22 studies; 111 dietary treatments for P). Only studies published after 2018 and focusing on the response to PhytM were considered. Model performance was assessed by calculating the root mean square prediction error (RMSPE, expressed as % of observed mean). The AD of PP (observed mean = 58 ± 34 vs. predicted mean = 63 ± 33 %, RMSPE = 11.8 %) and P (57 ± 17 vs. 63 ± 16 %, RMSPE = 21.2 %) were slightly over-predicted, whereas Ca AD was slightly under-predicted (63 ± 15 vs. 60 ± 11 %, RMSPE = 18.3 %). The simulated dose–response to PhytM indicated that supplementation with 300, 500, 1000, 2000, and 4000 FTU/kg released 0.7, 0.8, 1.5, 1.8, and 2.0 g of total Ca, respectively, assuming a Ca digestibility coefficient of 60%. The proposed model helps identifying key drivers of Ca-phytate interactions, phytate degradation and Ca and P absorption. The model provides a basis for future integration with post-absorptive metabolism to account for mineral homeostasis and homeorhetic regulation.

Meta-analysis of urinary calcium and phosphorus excretion as predictors of mineral status and dietary imbalances in growing pigs

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Kidneys are major regulators of calcium (Ca) and phosphorus (P) homeostasis by excreting the excess of absorbed minerals over effective retention in the body. As a result, urinary excretion of Ca and P, as well as their ratio, have been proposed as non-invasive indicators of mineral status. However, the large variability observed in urinary mineral excretion in pigs highlights the need to better quantify the nutritional factors modulating these responses. The objective of this meta-analysis was to assess the impact of dietary Ca, standardized total tract digestible P (STTD P) levels, and microbial phytase (Phytm) on urinary excretion of Ca, P, and their ratio. Sixty-five papers published between 1974 and 2025, including 70 experiments with 390 treatments, in which total daily urinary mineral losses were measured, were included in this study. Pigs body weight varied from 6 to 60 kg. Dietary STTD P and Ca were recalculated based on NRC (2012) feedstuff tables and were expressed in % of requirements using NRC (2012). All statistical analyses were carried out using GLM procedure of Minitab software, including experiments as a fixed effect. Results showed that urine Ca increased linearly ($P < 0.001$) and quadratically ($P < 0.001$) when dietary Ca increased. However, the losses of Ca in urine are influenced by STTD P (STTD P, STTDP x STTD P, STTD P x Ca, $P < 0.001$); when STTD P is below the requirement, increasing Ca results in higher losses because Ca cannot be deposited into bone. Urinary P excretion increased only quadratically ($P < 0.001$), showing really low losses below STTD P requirements, which increased with dietary STTD P due to excess for retention into bone and especially when Ca is below requirement (Ca x Ca, Ca x STTD P, $P < 0.001$) because of a lack of Ca to retain P into bone. Results showed that at low STTD P, urinary P is negligible and increases rapidly once requirements are met. Urinary Ca increased linearly with dietary Ca but then reached a plateau and subsequently decreased when Ca intake exceeded requirements, suggesting the formation of insoluble complexes that reduce Ca absorption. The Ca:P ratio in urine increased linearly ($P < 0.001$) and quadratically ($P < 0.001$) when dietary Ca increased and decreased linearly when increasing STTD P ($P < 0.001$), especially when STTD P is below the requirement (Ca x STTD P, $P < 0.001$). Increasing phytase decreases the Ca:P ratio in urine ($P < 0.001$). The highest ratios were obtained in pigs fed below STTD P requirements while the Ca requirement was fulfilled. These findings confirm that urinary mineral concentrations depend on the balance of the supply of dietary Ca and STTD P, as well as phytase supplementation. The information generated will be useful in ensuring that growing pigs receive adequate levels of Ca and STTD P, which are essential for proper bone development and the optimization of P utilization.

Health and management of unweaned calves on conventional and regenerative dairy farms

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Regenerative agriculture aims to increase soil health and biodiversity, enhance ecosystem services, and contribute to a more sustainable food production. In dairy farming regenerative practices include for example maintaining species rich grassland, sowing mixed herbal leys (mixture of grass, legumes, and herbs), applying solid manure and not using artificial fertilizers, limiting the amount of external feed input, and applying rotational grazing. Regenerative practices are also expected to improve animal welfare, including a positive effect on calf health. However, it is unknown whether these calves are managed differently than calves on conventional farms, and to date no studies have been conducted to assess the health of calves on such farms. The aim of this study was therefore to map calf management and living conditions and to assess the health of unweaned calves on regenerative and conventional dairy farms. Eight conventional and eight regenerative (of which six organic) Dutch dairy farms (mean ± standard deviation: 152 ± 70 versus 79 ± 38 calves born per year), which voluntarily participated in this study, were visited in the autumn of 2025. Farmers were first interviewed about calf management, whereafter unweaned calves were assessed for seven animal-based clinical health indicators, with a simple 'yes' or 'no' abnormality observed, using a predefined protocol. Additionally, four environmental elements were assessed for cleanliness. Calves were housed individually or in groups. Numerical data were analysed with Mann-Whitney-Wilcoxen test, whereas observed frequencies of animal (individual level) and environmental-based data (pen level) were analysed with either Chi-square or Fisher exact test. The conventional farms had a median of 16.5 (range 9-23) unweaned calves and the regenerative farms of 11 (range 4-20) either in individual (68 vs. 38 calves) or group housing (65 vs. 53 calves). Two conventional and 7 regenerative farms fed whole milk (5.7±1.1 L/calf/d), the others fed milk replacer (7.2±1.6 L/calf/d; $P<0.05$). Calves in individual housing had less space on conventional farms than on regenerative farms (1.5±0.4 vs. 3.5±1.4 m^2; $P<0.05$), whereas no difference was found for group housing (on average 4.3±2.7 m^2 /calf). Preliminary results showed a farm effect for all clinical health indicators. Nasal discharge was found less on conventional than regenerative farms (0.8% vs. 5.6%; $P<0.05$), and calves tended to be less responsive on conventional farms than on regenerative farms (12.1% vs. 4.5%; $P=0.05$). No statistical differences between the two systems were found for cleanliness of carpal joints, diarrhea, hairless spots, ocular discharge and lesions (average over both systems: 15.6%, 15.9%, 12.0%, 9.4%, 3.4% of the calves). Calf mortality (6.1%), calf weaning age (10 weeks) and cow replacement rate (29%) were also similar for both systems. With respect to cleanliness of environmental elements, conventional farms tended to have more dirty feeding troughs (52.3 vs. 25.0%; $P=0.06$) and milk feeding equipment (71.7 vs. 54.8%; $P=0.09$) than regenerative farms. No difference was found for cleanliness of drinking water facilities and of the floor within the pens (on average over both systems 67.8% and 49.7% was clean). Based on the limited number of farms included in this study, it seems that the variation in calf health and management is larger within than between the conventional and regenerative systems.

Implications of birth order on piglet survival

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Larger litter size usually results in longer farrowing duration which is often linked to piglets suffering from asphyxia during birth, compromising their viability. We assessed the relationship of birth order with key pig viability indicators: birth weight, 24-hour growth (as a proxy for colostrum intake) and survival. We hypothesised that pigs born later in the farrowing process would have higher mortality and lower early growth. We collated data from five studies in which we recorded birth order (n=2016), birth weight (n=2016), weight at 24 hours (n=1960) and at weaning (28 days, n=1792), rectal temperature (RT) at 24 hours (n=1186), survival/mortality (n=2016). All sows (n=141) were Landrace x Large White and all the studies were conducted in our pig research farm, in the same facilities and under similar management. Based on their birth order, we classified piglets as 'Early' (pig born 1st to 5th), 'Middle' (born 6th to 10th) and 'Late' (born ≥11th). Based on their survival, we classified piglets as 'Stillbirth' (died during farrowing), 'Early death' (died within 24h), 'Late death' (died before weaning), 'Post-weaning death' (died after weaning) and 'Alive' (survived the rearing period). Statistical analysis was done in Genstat (Version 24.1). For weight at birth, 24h and weaning, RT at 24h, birth-24h growth, 24h-Wean growth, separate two-way ANOVA was conducted with birth order and live status as main effects. Statistical significance was determined if P<0.05. Birth weight of piglets did not differ based on birth order (av. 1.33 ± 0.018kg, P>0.05) nor did weight at 24h (av. 1.40 ± 0.021kg, P>0.05) or at weaning (av. 8.11 ± 0.110kg, P>0.05). However, body weight gain in the first 24 h was higher in 'Early' compared to 'Late' piglets, but no different to 'Middle' piglets (0.073a, 0.054ab, 0.048b ± 0.0065 kg; respectively, P<0.001). There was no birth order effect on RT at 24h (av. 37.7 ± 0.06ºC, P>0.05). Overall survival for 'Early', 'Middle' and 'Late' piglets was 83%, 85% and 77%; respectively. 'Alive' piglets had higher weight at birth (1.38 vs. 1.11 ± 0.031kg, P<0.001), weight at 24 h (1.45 vs. 1.12 ± 0.038kg, P<0.001) and RT at 24 h (37.71 vs. 37.06 ± 0.066ºC, P<0.001) than piglets that did not survive. Among the piglets that didn't survive, 'Post-weaning death' were heavier than 'late death' piglets (1.216 vs. 1.054 ± 0.0528 kg, P<0.05), but no other differences were observed. On average, both 'Early death' and 'Late death' piglets lost weight during the first 24h (-0.035 and -0.009 kg, respectively), being significantly lower (P<0.001) than 'Post-weaning death' and 'Alive' pigs (0.060 and 0.069 kg, respectively). When we investigated the distribution of mortality across 'Early', 'Middle' and 'Late' born piglets, Stillbirths were 0.8%, 1.5% and 5.2%; 'Early death' was 4.1%, 2.6% and 7.5%; 'Late death' was 7.9%, 7.6% and 7.1%; and 'Post-weaning death' was 4.2%, 4.6% and 3.3%; respectively. Birth order had no relationship with birth weight although birth weight was related with survival, with pigs that died having lower weight at birth. Although not significant, piglets born 'Late' had 6 to 8% less overall survival rate. Birth order was related with body weight gain in the first 24 h, suggesting that piglets born 'Early' have higher colostrum intake. Meanwhile, piglets that died after farrowing and during lactation had little to no weight gain in the first 24h. Longer time spent in the birth canal and reduced colostrum intake could explain the higher values in stillbirth and early deaths observed among pigs born 'Late' in the farrowing process.

Risk factors in fattening rabbit housing: The role of animal density and bedding

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Large scale rabbit meat production is very demanding, and animal losses are a major problem in Switzerland, despite high husbandry standards. Some Swiss animal welfare labels as well as the public demand group housing and environmental enrichment including adequate bedding. However, group housing and bedding may increase animal dirtiness and infection risk. The type of bedding can affect hygiene, ammonia levels and mortality and should therefore be carefully considered. Animal density plays a critical role, as increasing density may improve economic efficiency, yet it simultaneously elevates pathogen pressure and the potential for disease spread. This experimental study was aimed at gaining a better understanding of how bedding type and animal density influence disease and mortality in group housed fattening rabbits in view of improving their health and wellbeing and reducing medication (especially antibiotics) while complying with Swiss animal welfare labels. We hypothesized that bedding type and animal density interact and that wood shavings and lower density lead to better health and lower mortality compared to straw and higher density. To this end, the 2 most popular bedding materials, straw and wood shavings were tested across 3 population densities in a completely crossed design on health and performance parameters of fatteners. For this, the maximum density allowed by the Swiss animal welfare ordinance (0.15 m²/animal) with 25 animals per pen, the maximum density allowed by the label IP-Suisse (0.25m²/animal) with 15 animals per pen, and a lower density (0.39 m²/animal) with 10 animals per pen were compared. Three trials with 2 groups of each of the 6 different treatment combinations were conducted, resulting in 12 groups per trial and 6 groups per treatment combination. Data was collected according to predefined protocols and analyzed using R. Survival analysis was applied to assess mortality, mixed-effects models were used for slaughter weight and weight gain, ammonia levels were compared using a t-test, and a generalized linear model was fitted for carcass section outcomes. The results showed no differences in mortality between the groups, with rates within the expected range according to the national average. Weight gain and slaughter weight were increased in groups of 10 animals compared to groups of 25 animals. Ammonia levels did not differ, but visual inspection of bedding material indicated that wood shavings were associated with lower moisture levels and reduced mold formation. Necropsy revealed that the intestines were more affected than the lungs, regardless of the group. However, bedding type influenced the extent to which the lungs were affected compared to the intestines, with straw having a worse impact on the lungs. These findings suggest that, within the tested density range, stocking density may not be a critical factor for survival, while other management or environmental factors may be more important. However, they indicate that stocking density and litter quality may affect the clinical condition of the animals and contribute to the cause of mortality, while wood shavings may help maintain a drier and less harmful microenvironment compared to straw. Furthermore, animals housed at lower densities may achieve a higher final weight.

Effect of group housing on behaviour and welfare of rabbit does in Mediterranean farming conditions

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New EU regulation on rabbit farming will likely establish group-housing requirements for growing rabbits to improve animal welfare. However, its applicability to rabbit does remains uncertain, particularly under Mediterranean farming conditions. Part-time group housing has been associated with chronic stress, increased aggression, and injuries in does. Moreover, part-time group housing is labor-intensive, which may limit its practical adoption by farmers. The study evaluates the feasibility of full-time group housing for rabbit does at 4, 6, or 8 does/group, and assesses the impact of group establishment timing (mid-gestation or during rearing). Three experimental blocks of 20 nulliparous does were used. In Block 1, does were divided into 3 groups formed at mid-gestation: G1 (8 individual does), G4 (4 does/group), and G8 (8 does/group). In Blocks 2 and 3, does were divided into one G1 group and two G6 groups (6 does/group), formed during rearing. Blocks 1 and 2 were inseminated, whereas Block 3 used natural mating. "A" and "B" refer to insemination events. Weekly welfare assessments included skin injury scores (0=no wounds to 3=bleeding wounds), presence of hairless areas (≥2 cm), and pododermatitis degree (0=healthy pad to 4=open wound). Ear wounds were recorded when >2 cm. Behavior was evaluated daily using 5-min focal sampling of the group, 3 times/d. Group fertility was calculated as (number of births/number of inseminations × 100). Data were analyzed using Kruskal-Wallis test for ordinal variables and the Chi-squared or Fisher's exact test for categorical variables. In G1, fewer injuries were detected than in group housing ($P<0.05$), but stereotypies (cage biting, scratching at the feeder, pulling hair) were observed ($P<0.05$). In G4, does presented moderate injuries (score 1), which healed quickly without intervention; fights occurred sporadically, mainly causing minor ear-tip wounds. In G8, fight frequency and intensity increased over time ($P<0.05$), causing severe ear wounds (score 3) in 2 rabbit does, which required ending the experimental block. In G6 (formed during rearing), rabbit does showed more affiliative behaviors (body contact, nose-body contact, sniffing, nose-to-nose contact, grooming, and sleeping together) than G4 and G8 ($P<0.05$). Only one G6 group displayed agonistic behaviors requiring the separation of 2 does. Stereotypes were not recorded in any group-housing treatment. In all group-housing groups displayed some agonistic behaviors (chasing each other, biting). Fertility was similar among experimental groups within blocks and insemination/mating event (Block 1A, 80%; Block 2A, 50%;Block 2B, 62%; Block 3A, 83%), except for the second insemination in Block 1, where individual cages had a greater fertility (100%) than group-housing (0% and 12.5%; $P<0.001$). Natural mating resulted in higher fertility (82%) than insemination (58%; $P=0.021$). In both G8 and G6, two does shared a nest without triggering aggression or reducing kit survival, and the number of kits weaned did not differ among blocks (Block 1, 8±1.5; Block 2, 10±1.1; Block 3, 11±0.89; $P>0.05$). In conclusion, forming stable groups during rearing promotes better socialization, reduced aggression, and increases affiliative behaviors, thereby improving welfare and potentially fertility. Fertility is also improved by natural mating. Nest sharing was not problematic, although nest-box dimensions warrant reconsideration. A group of 6 does (0.4 m²/doe) appears suitable for maintaining good welfare, although further studies are needed.

Effects of housing and management strategies on productivity and welfare of young rabbit does

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Under standard farming practices, crossbred young rabbit females replacing culled does are moved to the farm between 10 and 14 weeks of age and housed in individual cages. During the rearing period, young females are fed commercial diets characterised by moderate energy and high fibre concentrations, like those administered to growing rabbits during the post-weaning period, and then mated for the first time at 18–20 weeks of age. Few studies have evaluated the effects of the diet and the age at first insemination on the live weight at insemination and reproductive performance during the first pregnancy and kindling. In addition, in a context where public opinion and European legislation promote group housing of both growing and reproducing rabbits, the effects of group housing on the welfare of young nulliparous does need to be explored. Thus, this study evaluated the effects of housing (single vs. paired), age at first insemination (19 vs. 21 weeks), and diet (F, high fibre vs. S, high starch) in 192 crossbred rabbit does (Hyplus Grimaud Frère) allocated at 14 weeks of age to eight experimental groups in a 2 × 2 × 2 factorial arrangement (2 group size × 2 ages × 2 diets). The diet F was characterised by 24.5% ADF and 5.7% starch, whereas the diet S by 21.0% ADF and 8.4% starch. During the 7 weeks of growth, housing in pair reduced body weight at the first insemination (−4.7%; P<0.01), daily growth rate (−21%; P<0.001), and feed efficiency (−20%; P<0.001). Fertility rate was lower in rabbits housed in pair (74.7% vs. 86.3%; P<0.05), while weight gain during pregnancy (222 vs. 65 g; P<0.001) and live-born kit weight (59.3 vs. 55.3 g; P<0.05) were higher. Delaying first insemination from 19 to 21 weeks increased doe body weight at first insemination (+5.4%; P<0.001) and fertility rate (74.4% vs. 86.6%; P<0.05). During pregnancy, weight gain (243 vs. 46 g; P<0.001) and feed intake (−8.9%; P<0.001) were lower in does inseminated at 21 weeks of age compared to 19 weeks. The age at insemination did not affect lesion frequency and severity in pair-housed young does. Diet S enhanced daily growth rate (+18%; P<0.01) and improved feed efficiency (+25%; P<0.001) from housing to the first insemination compared to diet F. Conversely, the same diet S reduced feed intake during pregnancy (−6.9%; P<0.001) and the total weight of kits born alive (−11%; P<0.05) in comparison with diet F. In conclusion, pair housing of young does proved disadvantageous for both productivity and fertility. In contrast, delaying first insemination from 19 to 21 weeks increased fertility without negatively affecting growth performance, feed intake, and lesion occurrence in pair-housed young does. The administration of a diet richer in starch improved growth and feed efficiency, but decreased the weight of litter at kindling, without affecting doe fertility. Acknowledges: This research was funded by the European Union – Next Generation EU (PRIN 2022 “RABBINOUT”; CUP: C53D23005340006).

Acute ammonia concentration peaks present a delayed effect on reproductive and productive performance of rabbit does

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Intensive rabbit production under Mediterranean relies on highly environmental controlled buildings, thus being very sensitive to ventilation misfunctions. Ammonia (NH_3) is a key contaminant in livestock buildings and concentrations above 8–10 ppm are considered detrimental for animal health. The objective of this study was to quantify both immediate and delayed effects of an acute NH_3 peak on reproductive and productive parameters in a commercial rabbit farm. In this work, environmental variables were continuously monitored in a semi-intensive rabbit house equipped with mechanical ventilation and evaporative cooling. In July 2023, a short-term reduction in ventilation was produced due to a power failure. Consequently, NH_3 concentration increased from baseline values of approximately 4 ppm to average levels of 29 ppm, with short transient peaks reaching 58.85 ppm. Indoor temperature rose moderately from 25.5 to 29 ºC, while relative humidity remained high (around 90%), within the typical summer range for Mediterranean production systems. Then, several productive parameters were evaluated, both during the failure and during the following 6 weeks, including mating acceptance, fertility (positive palpation at day 12), abortion rate, stillborn percentage and mortality at weaning. Logistic regression was applied to dichotomous variables and T-tests to continuous outcomes. Fertility and abortion rate were not significantly affected by the NH_3 episode. However, significant alterations were observed in mating behaviour ($P<0.05$) and offspring viability ($P<0.01$). Does mated during the week following the NH_3 peak showed a clear reduction in mating acceptance, indicating a short-term carry-over effect after environmental restoration. A marked increase in stillborn rate was detected in litters born during days of elevated NH_3 concentration or immediately afterwards, reaching 42.5% in parturitions occurring just after the peak. Furthermore, a second increase in stillborn kits was observed at the end of August, corresponding to does mated during the NH_3 peak, suggesting impaired implantation or early embryonic development. This pattern indicates a delayed reproductive effect associated with exposure during the peri-implantation period. Mortality at weaning did not increase in kits weaned immediately after the episode. Nevertheless, a pronounced rise was recorded in animals that were six days old at the time of the NH_3 peak and in offspring from does mated shortly before or after the event, indicating increased vulnerability linked to early-life exposure and early gestational stress. These results demonstrate that short-term ventilation failures during summer conditions, such as those caused by brief power outages, may generate acute NH_3 peaks that do not necessarily affect fertility but can induce both immediate and delayed effects on implantation success, stillbirth rate and early kit survival.

Selected best practices for improving on farm welfare of rabbits: interrelationships with sustainability pillars

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Improving welfare and sustainability in rabbit farming is crucial to ensuring the future of this sector. However, to date, there is no structured knowledge linking welfare and rabbit farm sustainability. One of the key actions of the European Partnership for Animal Health and Welfare (EUPAW) is to review and identify best practices that enhance animal welfare. Then, the process has considered to evaluate how these practices interact—both positively (synergies) and negatively (trade-offs)—with the three pillars of sustainability: environmental (EV), economic (EC), and social (SO). To achieve this, dedicated working groups have been established for each major farm animal species, including rabbits. A scoping review of the available scientific literature was then performed using pre-selected string searches in Web of Science and Scopus for selected BP (tot=14, 9 on-farm, 4 during transport). The search was combined with a set of keywords related to sustainability, agreed upon and shared with all working groups and focusing on kits, growing rabbits and reproducing does as production categories. The aim was to identify both available knowledge and gaps in existing literature. All stages of the work were conducted in accordance with a Standard Operating Procedure (SOP) to ensure consistency and uniformity across the different species-specific research teams. The results were then collected and screened to exclude papers that were not relevant according to the SOP, as well as reviews and mini-reviews. The information contained in the remaining articles was extracted and cataloged to gather existing evidence on the relationship between the selected BP and each sustainability pillar. In this context, the methods employed to conduct the scoping review have been described, with preliminary results to be presented subsequently. As for rabbits, each BP will be introduced separately and the extracted information from each sustainability pillar will be discussed both in view of direct and indirect relationships with the BP. This work was co-funded by the European Union's Horizon Europe Project 101136346 EUPAHW.

On-farm hatching in broiler production: Field experiences with slow- and fast-growing genotypes in Germany

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On-farm hatching is an alternative hatching method where the hatching eggs are brought to the broiler house on approximately the 18th day of incubation and the chicks hatch between day 19 and 20. The chicks have direct access to feed and water, and there is no need for handling the chicks in the hatchery (for example separating from the eggshells, conveyor belts, packing in boxes) nor transportation. Furthermore, the chicks hatched in this way do not have to get used to a new housing environment, and possible temperature fluctuations from the hatchery through handling and transport to the broiler house are avoided, too. The aim of this practical study was to investigate the effects of on-farm hatching on animal health and welfare in fast and slow-growing broiler genetics. The investigations of chicks hatched on farm (OH) and in the hatchery (HH) took place on seven commercial farms; each farm raised OH and HH chicks in different houses at the same time. Four of these farms kept fast-growing broiler chickens (FG) (Ross 308 and Cobb 500) and three kept slow-growing genetics (SG) (Ranger Classic, Hubbard 757, and Hub-JA-Coloryield 57 Brown). A total of 52 OH fattening runs (37 FG and 15 SG) and 41 HH runs (26 FG and 15 SG) were monitored. In addition to performance parameters (hatchability, weight development, mortality), chick quality (Pasgar scores with evaluation of navel, beak, legs, belly, and reflexes; with a score of 5-10, where 5 indicates poor chick quality and 10 indicates chicks with no findings) on day 1, as well as antibiotic use were assessed. The results for hatching rate for OH-chicken (OH: 84.9% ±7.5 and HH: 84.0% ± 8.8), 7- day mortality (FG: 1.60% ±1.62 OH vs 0.74% ±0.84 HH and SG: 1.16% ±0.98 OH vs. 1.16% ±0.72 HH) and total mortality (FG: 3.31% ±2.18 OH vs. 2.55% ±1.70 HH and SG: 2.91% ±1.29 OH vs. 2.79% ±1.25 HH) for both slow- and fast-growing genetics were comparable to the chicks hatched in the hatchery (all $p>0.05$). OH-chicken were significantly havier on d 1 (FG: 58,7g ±12,0 OH vs. 55,5g ±11,6 HH and SG: 51,2g ±7,2 OH vs. 49,8g ±5,7HH) ($p<0,001$) but had lowerchick quality (FG: 8.91±0.90 OH vs. 9.21 ± 0.86 HH and SG: 9.48 ± 0.67 OH vs. 9.72 ± 0.50 HH) ($p< 0,001$). The frequency of antibiotic treatment between the hatching forms (OH 13.3% vs. HH 13.3%) in SG was lower than in FG (OH 89.2% vs. HH 92.3%), with a tendency to more antibiotic use in FG for HH-chicken. The field study show that on farm hatching can be an alternative to conventional hatching in the hatchery for fast and slow-growing broilers without adverse effects on production and health parameters. The effect of on-farm hatching on the stress levels of the animals is the subject of further investigation. This work (Model- and Demonstration Project for animal welfare) is financially supported by the Federal Ministry Agriculture, food and Regional Identity based on a decision of the Parliament of the Federal Republic of Germany, granted by the Federal Office for Agriculture and Food (FKZ: 2820MDT240).

Minimal evidence for locomotor imitation in pullets: Insights from a ramp vs. jump two-action procedure

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Social learning can shape foraging and resource use in chickens, but the evidence for copying specific locomotor actions is limited. We used a two-action procedure (ramp vs. jump) to examine if pullets copy an observed ascent method demonstrated by same-age birds when both options provide access to a platform with rewards. This approach was intended to assess action matching while limiting alternative mechanisms such as social facilitation and local/stimulus enhancement. Observer pullets were assigned to one of three treatment contexts: 1. Trained demonstrator (conspecifics trained to ascend via ramp-left, ramp-right, jump-left, or jump-right), 2. Untrained demonstrator (arena acclimated but untrained, free to choose ramp or jump), and 3. No demonstrator. Day-old white-feathered female chicks (n=48) were housed in six floor pens (12 birds/pen) with a 15 cm high, 150 cm long perch and water drinker lines positioned at bird eye-height, but no other elevated structures. Prior to testing, all chicks were familiarized with the test arena to reduce novelty effects. At 7 weeks of age, birds viewed the assigned context on 5 consecutive observation days in a test arena with a start box, a 40 cm high platform, and a 15 cm wide, 65 cm long ramp set to 50°. This set-up ensured ramp use and jumping were comparable in potential energy gain and likely to involve wing use. Before each session, observers were individually shown and allowed to consume a high-value reward (sweet corn, raisin, mealworm mix) and to join their conspecifics in a crate (social attraction). On day 5 of observations, each observer was tested individually: the same luxury reward/social attraction was shown beforehand, birds were released from the start box and given 5 minutes to reach the platform via ramp use or jumping while the food reward and conspecifics were present on the platform. Data were analyzed using a generalized linear mixed model (SAS), with demonstrator treatment as a fixed effect and pen as a random effect. Fisher's exact test was used for sparse sub-groups. Out of 48 pullets 19 reached the platform (39.6%). Of the birds that reached the platform; 45.7% had no demonstrator, 37.5% trained demonstrator, and 37.5% untrained demonstrator, with treatment having no effect on platform reaching ($p = 0.8843$). Thus, most pullets did not reach the platform, and demonstrator exposure had no overall effect. Few birds jumped to reach the platform (2/19; 10.5%). Pullets that observed a trained demonstrator ($n = 12$) could choose to copy the ascent method shown. While jumping was rare, observing a trained ramp demonstrator led to more pullets copying ramp use ($p = 0.0101$). This suggests copying within this subgroup, but the effect is limited in scope and sample size as only 5/48 (10.4%) reached the platform and matched the trained demonstrator's method showing minimal locomotor imitation under the present conditions. This indicates that young pullets primarily rely on individual learning when tasks are readily discoverable. When copying occurs, it emerges only in the lower-risk ramp action suggesting that individual exploration, not same-age demonstration, is the dominant mechanism shaping how young birds learn to navigate 3-D space.

Opinion of the FRCAW on the impacts of loading density on fall and injury risks in cattle and pigs, and on piling-up behaviour and injury risks in poultry

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In 2023, the European Commission published a proposal for a new regulation on the protection of animals during transport, aiming to repeal Council Regulation (EC) No 1/2005. This draft regulation seeks to increase the space allocated to animals, based on the recommendations of the EFSA scientific opinions on animal welfare during transport, which cover pigs, cattle, domestic birds, rabbits, equids, and small ruminants. At the request of the Animal Welfare Office (BBEA) of the General Directorate for Food (DGAL) at the French Ministry of Agriculture, the French Reference Centre for Animal Welfare (FRCAW) studied whether the loading densities proposed in the draft regulation led to more falls and injuries during transport. The FRCAW focused its analysis on three specific groups of species – pigs, cattle, and poultry- through three dedicated reports. These reports summarize the key points of the EFSA opinions, with a particular emphasis on falls, piling-up behaviour (in poultry), and injuries associated with loading density during transport. Additionally, the FRCAW reports provide an in-depth analysis of the scientific literature -primarily sourced from the Web of Science – on the impacts of varying loading densities and space allowances on critical welfare indicators, including behaviour, physiology, mortality, and carcass quality. They also identify the factors that increase the risk of falls and injuries in cattle and pigs during transport and propose strategies to mitigate these risks. However, few studies have specifically examined animal falls, piling-up behaviour in poultry, or injuries at the space allowances proposed in the new regulatory framework. As for the key findings, in pigs, no evidence suggests that the proposed loading densities would increase the risk of falls or injuries. Some studies indicate that space allowances similar to those proposed in the draft regulation are associated with fewer haematomas and shorter fight durations (a known cause of injuries). Increased space also enables pigs to sit or lie down, further reducing fall risks. In cattle, current evidence indicates that the space allowances proposed in the new regulation are associated with fewer injuries in calves compared to the current standards. For adult cattle, the single comparative study available shows that injuries are reduced under the proposed allowances, without any significant change in the number of falls. Importantly, no studies suggest that the new space allowances would worsen fall or injury risks compared to the existing regulatory requirements. In poultry, greater available space (floor area and crate height) mitigates the negative effects of restricted movement, although increasing floor area alone may correlate with a higher incidence of injuries—though the underlying causes remain unclear. Temperature, time spent in crates, and bird-catching methods are identified as additional factors influencing the impact of loading density. In conclusion, while current findings suggest potential welfare benefits from increased space allowances, further research is needed to confirm these results. Since animal welfare during transport is influenced by multiple interacting factors, reducing falls and injuries requires a holistic approach. Key parameters to consider include driving quality, route conditions, vehicle design, in-vehicle environmental conditions (temperature, ventilation), as well as journey duration and distance.

Catching and loading of end-of-lay laying hens: effects of body position on endocrine and respiratory stress responses

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Depopulation of end-of-lay laying hens represents a critical phase with regard to animal welfare. In Europe, hens are commonly caught by one leg and carried inverted, a practice that may induce anxiety, respiratory impairment and physiological stress. Current EU legislation prohibits handling methods that cause unnecessary pain or suffering, yet scientific data quantifying the physiological burden associated with different catching techniques are scarce. Upright catching, in which hens are carried in a vertical position with the wings restrained against the body, has been proposed as a welfare-friendly alternative, but its physiological impact has not been sufficiently evaluated, yet. Therefore, the aim of our experimental study was to quantify and compare the physiological stress responses of laying hens during depopulation using two different catching methods (inverted vs. upright) under controlled conditions. Special emphasis was placed on acute endocrine stress reactions and potential respiratory impairment during handling. In total, 100 Lohmann Brown end-of-lay laying hens (110 weeks of age) were divided into ten experimental groups with 10 birds each. Eight groups were handled under experimental conditions simulating commercial depopulation, two groups served as controls (N=20 hens per treatment/control). Birds were caught either conventionally by both legs and carried inverted or caught upright using both hands around the abdomen with wing fixation. Handling durations of 5 s (typical commercial practice) and 60 s (upper limit defined in EU Regulation 1099/2009) were applied. Acute stress responses were assessed by collecting blood samples two minutes after handling to measure plasma corticosterone concentrations and heterophil-to-lymphocyte ratio. Acoustic cardiorespiratory signals were recorded using an electronic stethoscope during handling. Heart rate ranged from 126–578 bpm in upright hens (272 ± 164 bpm) and from 147–567 bpm in inverted hens (367 ± 180 bpm). Inverted hens showed elevated cardiac activity and shorter inter-beat intervals (213 ± 109 ms vs. 285 ± 120 ms), indicating increased cardiovascular activation. To avoid confounding effects of pre-slaughter fasting, birds were not feed-deprived prior to handling. The same experimental procedure was applied to end-of-lay laying hens of a white genotype. The study provides comparative data on endocrine stress indicators and respiratory responses associated with inverted and upright catching methods. Differences between handling durations and genotypes further elucidate factors influencing stress susceptibility during depopulation. Analyses are ongoing and results will be presented at the conference. The results contribute to evidence-based recommendations for welfare-oriented depopulation practices and support refinement of current handling procedures in commercial aviary systems.

Comparative assessment of euthanasia methods for RNA integrity in rainbow trout (Oncorhynchus mykiss) larvae

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In the European Union, research involving fish is governed by animal welfare regulations. The onset of independent larval feeding marks the crucial developmental stage at which Directive 2010/63/EU on the protection of animals used for scientific purposes applies. Earlier developmental stages are generally exempt from this directive, provided the larvae are euthanized before reaching this point. However, standardized euthanasia protocols for larvae are lacking, namely due to differences in overall sensitivity compared to adults and between species (e.g. zebrafish and rainbow trout). This raises animal welfare concerns regarding the humaneness of common euthanasia methods, as well as their potential to compromise mRNA expression and integrity, thereby limiting suitability for transcriptomic analyses. The present study therefore aimed to identify a suitable euthanasia method for cold-water fish larvae that ensures both animal welfare and scientific reliability. This study evaluated the effects of three commonly used anesthetics, each applied at two concentrations, on RNA yield and integrity in rainbow trout yolk-sac larvae: tricaine mesylate (MS-222; 5 g/L [MS5], 10 g/L [MS10]); clove oil (eugenol; 2 mL/L [E2], 4 mL/L [E4]); and 2-phenoxyethanol (10 mL/L [PE10]), 15 mL/L [PE15]). Five larvae of each euthanasia group and two control groups (killed in liquid nitrogen [LN2], and by decapitation respectively), were used for RNA analyses. RNA extraction was performed with a standardized method using a commercial kit. The RNA yield was determined at a wavelength of 260 nm, and purity was assessed by absorbance at 230 and 280 nm. The RNA integrity number (RIN) and S28/S18 rRNA ratios were obtained using microchip electrophoresis. RNA extraction yielded 29.4 – 162 µg total RNA, with A260/A280 ratios of 2.05 – 2.18 and A260/A230 ratios of 1.79 – 2.37 across all euthanasia methods. RIN values ranged from 7.3 – 8.7 with the LN2 group being the lowest and E4 group the highest. The S28/S18-ratio varied between 1.14 – 1.76. RIN strongly correlated with the S28/S18-ratio ($r = 0.84$; $p < 0.001$), and moderately with total RNA yield ($r = 0.6$, $p < 0.001$). A260/A280 showed negative correlations with RIN ($r = -0.49$, $p < 0.001$) and A260/A280 ($r = -0.34$; $p < 0.05$). No significant differences were observed regarding RNA yield and sample purity (freedom from protein contamination, i.e. A260/A280 ratio). However, RNA from the E4 group had significantly lower A260/A230 ratios compared to E2 ($p < 0.01$), LN2 ($p < 0.005$) and P15 ($p < 0.01$) groups. RIN and S28/S18 rRNA ratios were significantly reduced ($p < 0.05$) in LN2 and MS10 groups, respectively. These results demonstrate that the euthanasia method used significantly influences RNA integrity in rainbow trout larvae, even when total RNA yield and general purity remain comparable. Liquid nitrogen shock resulted in consistently lower RIN values, whereas selected anesthetic treatments preserved RNA integrity more effectively. Lower RIN and 28S/18S ratios in LN2 and MS10 groups indicate that rapid physical inactivation or high anesthetic concentrations do not necessarily ensure optimal transcriptomic quality. Consequently, euthanasia protocols for early life stages should consider species-specific physiology, developmental stage, and downstream molecular applications. Selecting and optimizing anesthetic type and dosage is therefore essential to balance animal welfare with the reliability of molecular data generation.

Do stray currents impact the behavior, health and performance of dairy cattle? The farmer's perspective

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Dairy cow performance, health and behavior are influenced by animal characteristics, herd management practices, and environmental conditions. Among these, stray currents are frequently reported as contributing to the deterioration of dairy cows' condition. Stray currents can originate from nearby energy infrastructures (e.g., wind turbines, antennas, high-voltage power lines), on-farm installations (e.g., solar panels, milking robots, electronic feeders, grid-powered electric fences), or from grounding and wiring faults in farm facilities. Stray currents generate voltages perceivable by living organisms, with dairy cows considered approximately ten times more sensitive to electrical stimuli than humans. Such exposure may affect behavior, health and performance. This study aimed to provide an overview of French farmers' perspectives on the perceived effects of stray currents on dairy cattle. Surveys were conducted across France by MSc students. Farms experiencing or suspecting problems with stray currents were recruited via social media and advisory organizations supporting herds with performance issues. In total, 36 dairy farmers participated in semi-structured interviews. Topics addressed included problem characterization, the sequence of events, electrical and energy infrastructure on or near the farms, and the perceived impacts on animal behavior, health and performance. Data were analyzed using thematic analysis and multiple correspondence analysis (MCA) to explore relationships between reported problems and electrical or energy sources. Results showed that 18/36 farms were equipped with more than four electrical devices. 16 farms were located within one kilometer of an external energy infrastructure, while 20 were located more than one kilometer away. All farmers reported behavioral changes and 33 reported performance issues. The main behavioral changes reported were agitation (23), refusal to enter the milking parlor (27), reduced water intake (21), as well as kicking and signs of stress (28). Among the health problems reported, increases in somatic cell counts (31), mastitis (21) and higher mortality rates (14) were identified. A decrease in milk production was reported on 26 farms. MCA allowed the identification of three profiles: the first was mainly associated with internal sources and combined problems of agitation, abnormal milking behavior, decreased milk production and increased mortality; the second concerned internal and external sources presumed by farmers to have a predominant impact on performance; and the third was linked to nearby energy infrastructure, combined with internal sources, mainly associated with behavioral changes. Overall, the study highlights farmers' concerns regarding stray currents and their potential impacts on farm animal behavior, health and performance. This study emphasizes the need for further research to better characterize the effects of stray currents and develop practical mitigation strategies to support sustainable dairy farming systems.

Assessing the effect of trees on the welfare of dairy cows by limiting insect pressure

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Insect pressure on grazing animals is increasing with global warming. However, using chemical products to protect grazing animals from this nuisance is not permitted on organic farms. Some trees have a natural repellent effect on insects and animals could benefit from these trees in agroforestry systems. In our trial, we assessed insect pressure on 6 herds of cows on pasture with/without trees insect traps (one H-trap in the meadow and sticky traps in various tree types per herd), observed defensive behaviours of cows for 5 minutes every week over 3 months and counted flies on the animals. Our results highlight that defensive behaviours of cows decrease in time for an individual on a specific pasture. This shows that cows get habituated by the insects around them and the defensive behaviours they express alone does not fully qualify the cows' welfare. To grasp which factors influence the total number of insects per cow, we modelled the number of insects at observation (reported from photography) depending on (1) the date, (2) the location on the pasture (close, under or no trees), the weather (sunny under shade or in the sun and cloudy), (3) the colour of the cow, (4) the air temperature (recorded ourselves for the temperature under trees and national database otherwise) and (5) at the lacrimal point of the cow (recorded with a infra-red camera). Only the weather variable and the date significantly influence the number of insects. The expected counts of insects on a cow are about 1.5× higher in sunny weather (regardless of whether the animals are in the shade or in the sun) than when the weather is cloudy. Additionally, the number of insects follows a quadratic formula with a negative value of the x^2 coefficient showing that the number of insects follows an inverted U form depending on the date between the beginning of June and the end of August. To further assess the dynamics of insects on pasture, we collected insects with sticky traps under a tree and with the H-traps in the meadow. First, we highlight that the number of biting insects under trees was close to inexistant while we collected a high number of biting insects, especially horseflies, with the H-traps. To discriminate influencing factors, we performed a mixed linear model per trap type and observed that while temperature and months when the traps were hanged influenced the number of insects on sticky traps, only the location by canton influenced the number of insects collected in H traps. Our result shows a significant increase of the total number of insects collected in sticky traps with increasing temperature, lower insect counts under Pinales compared to Sapindales, and finally higher numbers of insects in June and July compared to August therefore suggesting a similar curve as the one observed of insect on the cows. Regarding the number of insects (biting and non-biting) collected in H traps, the pastures located in the hilly canton of Jura showed significantly more insects (biting and non-biting) than in the alpine canton of Schwyz. The results in the lowland canton of Aargau showed no significant difference compared to both Jura and Schwyz. To conclude, in a follow up experiment, e-DNA, to quantify the number of insects that landed on a cow over a large period of time, and behavioural camera could contribute to fill the gap as this dataset, even though large, does not cover continuously either the cows' behaviour or the insects that were landing on a cow.

Analysis of the behavior of finisher pigs in the 24 hours before death on a commercial farm

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Group housing systems where multiple pigs are reared together are common during the finisher period in commercial farms. In recent years, rising farm sizes have increased the number of pigs managed per worker. To improve productivity, it is essential to promptly detect and deal with problematic pigs at an early stage. However, under group housing conditions, it is difficult to monitor the behavioral features of individual pigs. This study aims to investigate the behavioral features of finisher pigs that died during the finisher period by analyzing video data from the 24 hours prior to death. This study included finisher pigs reared in a finisher barn on a Japanese commercial farm. Video data were recorded using ceiling-mounted cameras capable of capturing two pens simultaneously. We analyzed a total of 28 pigs, comprising 14 pigs that died during the observation period (death group) and 14 healthy pen-mates (control group). We analyzed the 24 hours prior to death, with each control pig assessed during the identical 24-hour time window as its paired deceased pig. We quantified the proportion of time spent in five postures (standing, sternal lying, lateral lying, sitting, and front-leg sitting), and the frequencies of feeding, drinking, and injurious behavior (being bitten). The chi-square test was used to compare the proportion of time spent in different postures between the groups. Temporal changes in feeding, drinking, and injurious behaviors were analyzed using Generalized Linear Mixed Models.The proportion of postures differed significantly between groups ($P < 0.05$). The death group showed lower proportions of standing and lateral lying, but higher sternal lying than controls. In the death group, the proportions of standing and sternal lying peaked 24–18 h before death and reached their lowest levels at 12–6 h, while lateral lying increased toward death. Feeding frequency was significantly lower in the death group ($P<0.01$), with a sharp decline starting 12 h before death ($P<0.05$). For injurious behavior, pigs in the death group were bitten significantly more often ($P<0.05$). A group-by-time interaction ($P<0.05$) revealed that being bitten intensified in the final 6 h ($P<0.05$). While overall drinking frequency did not differ between groups, it significantly declined within the death group during the final 12 h compared to the 24–18 h baseline ($P<0.05$). This study revealed distinct behavioral features in finisher pigs prior to death, identifying a higher proportion of sternal lying, a marked decline in eating and drinking behaviors starting 12 h before death, and a surge in injurious behavior as critical indicators of mortality. Regarding the shift in the proportion of postures, particularly the increase in sternal lying, this likely reflects energy conservation or respiratory distress in moribund pigs. Significant reductions in feeding and drinking 12 h prior to death are critical signs of impending mortality. Interestingly, the lack of a group difference in drinking might be due to compensatory water intake in febrile pigs, masking the decline. Furthermore, the surge in injurious behavior in the final 6 h suggests that debilitated pigs become targets for pen-mate aggression due to their inability to retreat or resist. Integrating these parameters into automated monitoring systems could facilitate early intervention, improving farm productivity and animal welfare. This research was supported by a grant (Smart Agriculture Demonstration Project by Ministry of Agriculture, Forestry and Fisheries of Japan; Project No. Livestock-5H3) from National Agriculture and Food Research Organization (NARO).

The restraint enclosure effects due to African Swine Fever on the reproductive parameters, ethology patterns and welfare of boars and sows of the free-range Greek autochthonous black breed.

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Pigs have been raised in Greece since the Homeric era. Following World War II, the introduction to farmers of genetically improved and high yield European breeds and the initiation of intensive pig production had a negative impact on the production of free-range black pigs. In the following years the remaining herds of the autochthonous black-breed pigs were raised in pastures and oak-tree forests, confined in "primitive" infrastructures. Due to African Swine Fever (ASF), the swine producers have been forced to comply with strict biosecurity measures during the last five years. The aim of this study was to record and determine differences in the reproductive ethology patterns, reproductive efficiency and welfare status between the breeding stock that was raised in free range (reproductive parameter's record keeping data of two consecutive years prior to enclosure -PE) vs the breeding stock's reproductive efficiency data (reproductive parameter's record keeping data) following the transition time (TT) from the pastures to a total confined setting for a period of two years. Data was collected from three farms, located in three different Greek regions (farm A: Central Macedonia, farm B: Thessaly and farm C: Epirus, respectively). The farms are located in mountainous regions. The breeding stock capacity – sows and boars – of the three farms prior to enclosure were 160 – 12, 120 – 10 and 80 – 6, respectively, while during the two – year transition time the farms' breeding stock capacity were 130 – 10, 100 – 8 and 60 – 5, respectively. Sows were bred with natural service twice a year. The boars' ethology patterns and the libido (Lsc-in a scale of 1 to 5, with 1.: not mounting, 2.: mounting but no protrusion, 3.: delayed mounting followed by protrusion but no penetration, 4.: multiple mountings, delayed penetration – ejaculation, 5.: aggressive mounting – penetration, ejaculation) was evaluated at the peri-copulatory time. Breeding records were evaluated for wean to estrus interval (WEI) and conception rate (CR) in sows. In addition, the pre-weaning mortality rate (PWMR) was determined while the sows' ethological patterns during the peri-parturient time were evaluated. A two – sided Student's t– test was used for the analysis of data. P–values ≤ 0.05 were considered statistically significant. The results showed that the majority of the PE boars exhibited a similar ethological reproductive pattern (Lsc:5, 21/28 boars, $P \leq 0.05$), while the TT boars' ethology varied from lethargic (Lsc:1) to highly aggressive (Lsc:5 $P \geq 0.05$). Similarly, the PE sows had a higher CR and a normal WEI compared to the TT sows ($P \leq 0.05$, 85% vs 55%, 6d vs 11d). The strict restraint measures had a detrimental effect on litter size with PWMR reaching 45% ($P \leq 0.05$) due to crushing and in some cases primiparous sows exhibited cannibalism. The above results indicate that the long-term enclosure had a negative effect on the reproductive parameters and the ethology of the TT breeding stock, while it had a major negative impact on the overall welfare status of the farms.

New house, stressed cows? Behavioural observations following relocation to a new barn

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Regrouping is a common practice on dairy farms to adapt feeding and management cows' lactation status as well as milk yield, although changing the social environment might be stressful for the animals. In addition, the resulting social instability can have negative effects on their behaviour. This pilot study investigated if behavioural changes in dairy cows occurred after relocation to a new barn. Observations were conducted via video recordings in two compartments of a newly built free stall barn. Both recorded compartments kept a total of 24 animals and were identically designed (e.g. same drinking trough, cow brush) except for their different floors and lying areas (compartment A: deep straw; compartment B: slatted floor and cubicles). Beginning immediately after relocation, the behaviour of three focal animals (lactation 1-6) from each compartment was continuously observed for the first five days (milking times excluded). The observations focused on lying, eating and drinking behaviour as well as cow brush usage and agonistic interactions. For evaluation, it was examined if there were differences in the duration and frequency of the observed behaviours among animals kept on straw or slatted floor as well as among primiparous (pp) and multiparous (mp) cows. For all behaviours, the average duration as a percentage of the net observed time was calculated and their frequency was counted. The mean percentage of lying time across all observed animals was between 53.4 and 80.2% per day. No consistent trend in lying behaviour was found in the two different compartments. In compartment A, lying times first increased until the third day and then decreased (Mean: 70.5%), while in compartment B there were hardly any changes during the observation period (Mean: 68.6%). Contrary to expectations, the number of lying frequency also remained consistent over the observed period. Dairy cows in compartment A spent less time eating and drinking than those in compartment B. Regarding feeding behaviour, pp cows showed longer feeding times (Mean: 15.7%) than mp ones (Mean: 10.8%). Number of drinking frequency decreased after two days; this trend was observed in both compartments similarly. The cow brush was used more frequently in the straw compartment A than in compartment B, and the pp cows used it more frequently than the mp ones. Within the first 24 hours, 132 agonistic interactions with other cows were observed among the focal animals in compartment A. In contrast, there were 44 agonistic interactions in compartment B. After a decline on days 2 and 3, the total number of agonistic interactions in compartment A rose sharply until the end of the observation period. The longer lying times of cows in compartment A during the first three days indicated the high attractiveness of the comfortable deep straw bedding. The decline in drinking behaviour after the first two days might suggest that the initially increased exploratory behaviour reduced over time. The higher space allowance, respectively mobility, offered in compartment A led to a higher number of agonistic interactions on all observation days. Here, the particular increase on the fifth day after relocation was certainly because of one of the animals was in heat, which led to general unrest. To verify these first results, further investigations on the herd-synchronous behaviour of all cows in both compartments will follow. The project is funded by the Federal Ministry of Agriculture, Food and Regional Identity (BMLEH) based on a decision of the German Bundestag. The project is managed by the Federal Office for Agriculture and Food (BLE), FKZ: 28N1800003.

Does choice of calving location affect calving duration?

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Previous research studies showed that cows, particular primiparous, increase their distance to their group in the hours prior to parturition and that some cows prefer calving behind a hide if provided. Cows in group calving pens equipped with a blind may show less position changes during parturition and receive less prior attraction from pen mates. However, the use of a blind or secluded area for parturition varies to a large extent and is influenced by pen design and, e.g., parity or health status of the cow. Group calving pens with blinds, stocking density and preferred dimensions of blinds were previously linked with calving duration, but the relationship between the use of secluded areas and calving duration is not that clear. Thus, the objective of the study was to evaluate how the individual cow's choice to calve in a secluded area within a group pen is related to her calving duration. For this purpose, calving duration was compared to cows calving in the group area of the calving pen and to cows giving birth in an individual calving pen. Data on calving location and duration were collected in a barn with three group calving pens and four individual pens for calving at the research farm Karkendamm from August 2024 to September 2025. Each group calving pen comprised two secluded areas (3.50 x 3.45 m) with a blind height of 1.5 m towards the group area, the neighboring pen and the walkway. Four cows were kept together in a group pen from 2-3 weeks before the expected calving date (all-in/all-out; 21 m² per cow at full stocking density). After each calving, the respective cow left the calving pen. For the evaluation here, only calving events with four cows in the group pen were considered. Cows which gave birth in the individual pens were moved into the pen about 2-3 days before calving. The calving location and start and end time of calving was analyzed by video observations. Thereby, the calving duration was defined as the time duration from the beginning of observable abdominal contractions until the calf's hips were fully expelled. Altogether, 24 calving events (8 primiparous, 16 multiparous cows) from 24 group pen cycles were included in the analysis, and 39 cows (15 primiparous, 24 multiparous) which gave birth in individual pens. Differences in calving duration between pen type or calving location in the group pens were tested pairwise by Mann-Whitney U Test. Cows in individual and group calving pens had an average calving duration of 122 min (56-188 min, n=39) and 131 min (62-198 min, n=24), respectively. No difference was found with regard to pen type ($p = 0.3539$). Of the 24 cows giving birth in group pens, seven did choose the secluded area as calving location, with an average calving duration of 100 min (62-149 min, n=7), and 17 gave birth in the group area. Average calving duration of the latter was 143 min (75-198 min, n=17). Calving duration between the group pen locations differed significantly ($p < 0.01$). The shorter calving duration in cows which did choose to give birth in the secluded area is in line with previous reports on shorter calving durations in pens with a blind and low stocking density. However, it also contradicts earlier studies which did link health issues and thereupon based preferences for larger blinds with prolonged calving durations. In conclusion, pen design and group dynamics might influence both calving duration and individual cow's choice for or against secluded areas. The ongoing analysis thus intents to get a deeper insight into the question whether the reduced calving duration is linked to the feasibility to distance or hide from the group, or rather results from other causations such as parity, health and calf's breed or sex.

Opinion of the FRCAW on the Effects of Transport on the Welfare of Calves under Five Weeks of Age

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In 2023, the European Commission published a proposal for a regulation on the protection of animals during transport to replace Council Regulation (EC) No 1/2005. This new text proposes regulatory transport conditions based on the recommendations of the EFSA opinion on the welfare of cattle during transport, with specific recommendations concerning the transport of calves (EFSA AHAW Panel, 2022). The new regulatory proposal would increase the minimum age for the transport of calves to 5 weeks, whereas the minimum age is currently set from 10 days (for journeys over 100 km) to 14 days (for journeys lasting more than 8 hours). At the request of the Animal Welfare Office (BBEA) of the General Directorate for Food (DGAL) at the French Ministry of Agriculture, the FRCAW wrote a report addressing the main welfare issues encountered during the transport of calves under 5 weeks of age. The information reported by the EFSA has been supplemented by a comprehensive analysis of scientific literature not cited in the EFSA opinion, particularly studies that have been published since 2022. In Europe, calves are generally transported at the age of 2 to 4 weeks, a sensitive stage in their lives during which their own immune systems have not yet developed and their thermoregulatory capacities are limited. For such animals, transport conditions are likely to increase fatigue, stress, dehydration, the development of disease and mortality. This vulnerability decreases with age. Two-week-old calves are more vulnerable than four-week-old calves, who in turn are more vulnerable than six-month-old calves, whose immune systems have reached maturity. Furthermore, navel healing, which is a regulatory prerequisite for the transport of calves, is complete only at 3 to 4 weeks of age. Studies in the literature emphasise the need for good management of colostrum intake from birth to promote the transfer of passive immunity. They also stress that sufficient energy must be provided to calves by offering them milk replacer 4 hours before transport, and that the total journey duration should be limited to 8 hours. Altogether, current scientific literature recommends avoiding the transport of calves before four to four-and-a-half weeks of age. However, research on the impact of transport age on calf welfare remains limited. Future studies should systematically evaluate welfare indicators in calves aged 2 to 6 weeks – both transported and non-transported (control groups) – to fill this gap. Key research priorities include: nutritional and energetic needs of unweaned calves before, during, and after transport, as well as effective rehydration strategies; digestive impacts of consuming liquid or solid feed during transport; optimal rest periods during journeys for unloaded unweaned calves; development of an objective clinical scoring method to assess navel healing; effects of transport on calves' mental and emotional states.

Development of a research and demonstration barn to improve animal welfare in dairy husbandry and to regain societal acceptance

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A concept for a research and demonstration barn to improve animal welfare in dairy husbandry was developed, called "Future Dairy Barn" (FDB). Although the development and construction of the FDB were originally funded by the German government, the construction of the FDB could not be realized due to financial constraints and the FDB was built virtually. Housing conditions for dairy cattle have been improved in the last decades, for example leading to more loose than tethered housing. However, there is still a lot of room for improvement especially regarding animal welfare, societal acceptance and technical innovations, which do not compromise animal welfare but reduce the work load of the farmers and their employees. As animal welfare is a multidimensional concept that covers natural living and basic animal health and functioning, as well as the affective state, for the development of the FDB all three dimensions of animal welfare were taken into account. In the FDB, the cattle shall be able to (1) form a stable herd structure, (2) exhibit species-typical lying behavior, and (3) decide for themselves whether they stay indoors or outdoors. Referring to these three implications, the FDB concept integrates a family herd, which means that the calves can suckle their dams, heifers are reared within the herd and lactating as well as dry-off cows are kept together to avoid regrouping and to enable stable social relationships. A free lying area is included to allow species-typical lying behavior and pasture or paddock access is provided that the cattle can stay indoors or outdoors. A high degree of process automation is integrated in the FDB to reduce work load. Moreover, technical innovations like so-called "Smart Feeding Fences" and a "Smart Calf Sluice" are developed to realize the family herd concept. To meet the requirement of being a demonstration barn, a visitor corridor is included. Visitors can therefore look in detail into the FDB without compromising biosecurity. Future dairy production needs to be rethought. Society's demands on husbandry systems are increasing with respect to animal welfare of dairy cows. However, a higher workload of the farmers should be avoided. Therefore, investigations on long-term effects on animal welfare in a uniform social and housing environment with a high degree of automation techniques are needed. The FDB provides the opportunity to investigate holistic approaches on animal welfare in long-term studies and enables farmers and consumers to take a look into an alternative concept of dairy farming. The virtual application of the FDB available can be used to give an insight into the FDB and to transfer knowledge on animal welfare of dairy cows and their offspring.

Exploring the future of the European equine sector towards 2040: a participatory foresight approach

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A sector facing growing socio-environmental and economic pressuresThe European equine sector is facing increasing uncertainty driven by climate change, resource constraints, evolving societal expectations regarding animal welfare, and economic pressures. In this context, maintaining the sector's social acceptability and long-term sustainability has become a critical challenge. This study, developed by the European Horse Network (EHN), aims to explore how the European equine sector could evolve by 2040 under different socio-economic and environmental conditions. Its central research question is: How might the European equine sector evolve by 2040 under different economic, environmental, and social conditions, and what strategic actions are required to ensure its resilience and societal relevance? A participatory and matrix-based foresight methodologyA qualitative foresight methodology was applied, combining stakeholder workshops, expert interviews and a multi-country survey conducted across Europe. The study was structured using a matrix-based scenario approach, which consists in developing four contrasted scenarios based on the intersection of two key axes. In this case, the selected axes were (i) social acceptability of equine activities and (ii) sustainability, encompassing both environmental and economic dimensions. These two dimensions jointly reflect the sector's ability to maintain its "social licence to operate". The identified trends and drivers of change were then analysed and systematically projected across the four scenarios, allowing the exploration of coherent and plausible future pathways for the European equine sector. Four scenarios and the key conditions for the sector's futureThe main results highlight four potential futures: (1) strong societal integration of horses within sustainable rural development; (2) increasing competition for land and resources, requiring active defence of the sector's legitimacy; (3) marginalization of horses due to declining social acceptance and strict regulation; and (4) a contraction of the sector into an elite, exclusive activity. Across all scenarios, social legitimacy, environmental adaptation, and economic diversification emerge as decisive factors. The findings underline the growing importance of sustainability practices, welfare standards, innovation, and communication with the wider public. The study concludes that the future of the European equine sector is not predetermined but highly dependent on proactive adaptation. Strategic collaboration, improved policy engagement, investment in skills and research, and a clear demonstration of horses' societal value are essential to securing a resilient and socially accepted equine sector by 2040.

Professional Conceptions of Equine Wellbeing: Continuous Adjustment Grounded in Observation, Expertise, and a Human–Horse Moral Contract

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Debates surrounding equine wellbeing (EW) are increasingly frequent, fostering the development of a body of knowledge, of a growing market and questioning existing practices. Professionals are primarily responsible for finding an economic and ethical balance in their daily activities, which involve both caring for horses and making use of them. This research in management sciences aims to better understand how professionals conceive the wellbeing of the horses they live and work with and the debates on this topic. Twelve semi-structured interviews were conducted with six riding instructors and six racehorse trainers in France, selected for their diversity. A thematic analysis identified more than 380 meaningful semantic units, which were then hierarchized and reorganized to describe three main thematic axes related to EW. 1) The definition of EW according to professionals Professionals define EW through a human-centered approach closely linked to the notion of welfare. EW is associated with proper management of horses' living conditions, satisfaction of their fundamental needs, a respectful human–animal relationship, professional expertise, and the ability to interpret horses' subtle signals. They also point out a constant uncertainty, due to horses' silence and individual variability. Differences appear between instructors and trainers regarding housing systems, the relationship to work, and perceptions of horses' specific needs. Overall, professionals struggle to provide a unified definition of EW, which they perceive as complex and multifactorial. 2) EW as experienced by professionals in their daily work EW is experienced daily through an ethic of reciprocity, in which good treatment represents fair compensation for the work provided by horses, moreover because in some cases the pursuit of performance may threaten EW. Competence and expertise play a central role and rely on observation, interpretation, adaptability, and the ability to engage horses' cooperation. However, professionals emphasize the impact of economic, sporting, and institutional constraints, which require horses to work and be profitable, while limiting professionals' capacity to provide optimal living conditions. 3) EW consequences and challenges for the profession Professionals perceive EW as a recent and major social issue shaping the future of their professions, with trainers expressing greater pessimism. They view it as a cultural and societal dynamic and as a challenge to professional legitimacy. They agree that public debates are excessive, multifaceted, and overly polemical. They believe that public perceptions would improve if people observed everyday professional practices. Both groups highlight the key role of institutions in regulating this phenomenon. To conclude, the absence of a formal definition of EW reflects not disinterest but a pragmatic, individualized approach centered on human action. EW refers not only to the horse's subjective state but also to professional skills and continuous trade-offs between care, use, unpredictability, and economic constraints. While the scientific approach aims to abstract from diverse individual cases in order to establish general laws, professionals appear to favor a "science of the singular," adaptable to each horse and situation. In doing so, they highlight their expertise and provide valuable insights for better adapting public policies and professional training to the realities of the field.

The INOSYS portal, open access equine references

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The INOSYS portal for herbivore sectors (https://portail.inosys-reseaux-elevage.fr/FR/) was developed to address a growing need for reliable, comparable references to support the sustainability of European herbivore farming systems. In a context of economic pressure, environmental transition, and generational renewal, farmers and advisers require comprehensive and up-to-date technical and economic benchmarks to guide decisions at farm and sector levels. The portal provides open-access references for seven herbivore sectors, including cattle, sheep, goats, and equines. It currently hosts around 250 "case-type" farms—model farms representing coherent, well-documented production systems that are both optimised and realistic within clearly defined economic conditions. Among them, three concern equine "riding school" systems, with two additional equine case-types ("competition rider livery" and "trotter training stable") under integration. For each case-type, approximately 500 downloadable indicators describe the system's multi-performance, including economic viability, environmental performance, and labour organisation. For instance, recent analyses show that equine riding schools combining diversified income sources (lessons, boarding, and training) achieve higher income stability while maintaining comparable workloads. Sectoral dashboards aggregate these results by farm type and present consolidated indicators to facilitate comparisons. The portal also centralises all related publications, serving as a single access point for teachers, students, researchers, and policy-makers. By offering annually updated references and synthetic tools, INOSYS contributes to the design, evaluation, and dissemination of resilient and sustainable herbivore farming systems.

Cost of production: a management tool for equine businesses

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In a context of high market variability and increasing pressure on farmers' incomes, having reliable and comparable indicators is essential for effectively managing production systems. Widely used in the dairy and beef sectors, the national method for calculating the cost of production is currently being adapted to the equine sector. This management tool aims to improve understanding of the farmers' income levels and to identify opportunities for technical and economic improvement. This method, which has been developed within the INOSYS livestock farming networks since 2010, is recognized at both the national and international levels and is now widely implemented in France. By the end of 2024, more than 28,000 cost-of-production analyses had been carried out in herbivore production systems. It also provides a structured framework for advisors, strengthening their expertise and their ability to support improved efficiency. Although still little developed in the equine sector, this approach nevertheless represents a relevant lever for equine businesses. It offers an analytical framework that helps farmers step back from the operation of their businesses, compare their results with Equine Network benchmarks, and identify levers to improve income. For the past two years, methodological work has been conducted in France to adapt this national cost-of-production method to the equine sector. The study is based on the analysis of 145 farms from the Equine Network over four accounting years (2020 to 2023). The approach, conducted at the activity level, relies on allocating production resources, costs, and certain outputs among the different activities. Costs are expressed in production units (€/hour for riding schools, tourism, and horse-drawn transport, and €/day for horse boarding) and are then compared with revenues in order to assess each activity's ability to generate income for the farmer. Initial cost-of-production calculations were carried out for six systems (riding schools and equestrian farms, sport boarding and livery stables, trotting trainers and horse-drawn transport and driving). Average income levels vary depending on the system: below 1 minimum wage per full-time farmer in working livery, between 1 and 2 minimum monthly wages in riding schools, boarding livery and equine transport services, and above 2 minimum monthly wage in equestrian farms and trotting trainers. In riding schools in 2023, the cost of production, before the farmer's own remuneration for work, amounted to €14.99 per hour, compared with revenues of €19.55 per hour, resulting in an average income level of 1.5 minimum monthly wages per full-time farmer. Analysis shows that farmers achieving an income level of 2 minimum monthly wages are characterized by better generation of revenue, optimized use of the horse herd, and good alignment between labor and production resources. These initial results are encouraging for the sector. They provide benchmarks for production costs per activity and improve understanding of the key factors influencing income in equine businesses. Aligning the farmer's objectives with the production resources used is essential for the efficient operation of the business.

Ecosystem restoration potential of equine farms – development of a practical and scalable framework supporting the EU Nature Restoration Law

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The EU Nature Restoration Law highlights the urgent need to scale up ecological restoration opportunities across Europe's human-dominated landscapes. Agricultural land covers more than 45% of the EU's territory, and thus agroecosystems strongly shapes the fate of biodiversity protection. Among agricultural systems, the equine sector represents a land-use system of considerable extent, for instance in Central Europe, with 2.3 million horses across 87,000 equine facilities in France and Germany alone. Moreover, large-scale equine farms stand out as potential areas for restoration and biodiversity enhancement: they are often exempt from intensive production pressures and may maintain semi-natural habitats. Identifying the strengths and weaknesses of equine facilities in contributing effectively ecosystem restoration and facilitating biodiversity protection, requires a systematic assessment framework. In this study, we developed a Restoration Potential Index (RPI) for equine facilities. The index integrates patch-scale habitat heterogeneity, connectivity, ecosystem quality and topographic variation together with indicator species richness, and the proximity and size of (semi-)natural areas into a single standardized measure, assessed at three spatial scales. The RPI was applied in four sample areas (Germany; Austria; Hungary, and France) which differ in terms of management, habitat mosaicity and naturalness, but are comparable in terms of climatic conditions. Applying the RPI in these four case study sites showed clear comparability of equine yards with the index. Among the facilities, the Hungarian site stood out for its within-site heterogeneity, habitat patch quality, connectivity and species richness (each scoring the maximum value: 5). The French site showed low topographic variability (1) but compensated this with high (5) inner habitat connectivity, showing the importance of using several indicators to assess the restoration potential of equine properties. In the German and especially the Austrian site, indicators showed intermediate to lower within-site scores, except site-scale topographic variation in the Austrian equine yards (5), indicating a basis for potential restoration measures. The surrounding areas of each facility (except the Hungarian site) revealed potentially lower possibilities for species flow and habitat connectivity on the macrohabitat scale, and, except at the French site, they also lacked the presence of near-natural areas. Such lower scores of the close surroundings, and the complete absence of designated protected areas in the 5 km buffer around the equine yards may represent a challenge in restoration, yet underscore the importance of the equine facilities as potential biodiversity hotspots in highly exploited surrounding areas. The RPI provides a novel, transparent and scalable tool for evaluating the restoration potential of equine facilities and, if adapted, potentially other agroecosystems across Europe. We propose the integration of the RPI into management strategies at equine facilities and in future agri-environmental subsidy schemes, to support the involvement of private land in ecosystem restoration and rewilding, thus increasing the size of areas targeted by the EU Nature Restoration Law.

An Economic Overview of Equestrian Tourism in France

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Horse-related tourism activities contribute to the dynamism and attractiveness of French territories. These activities are diverse and widely dispersed, and there is limited data to objectively assess their economic impact. For this reason, the Economic and Social Observatory of the Horse (OESC) of the French Institute for Horse and Riding (IFCE), together with the IFCE Western Territorial Delegation, are conducting a national study, in collaboration with the French Equestrian Federation (FFE) and the National Federation of Horse Racing (FNCH), with the support of researchers in geography and economics. The objective is to identify and characterize horse-related tourism and leisure activities through key indicators (number of sites, attendance, turnover, full-time equivalent jobs, etc.). Data collection was carried out in late 2025 and early 2026 through internet research, online surveys, and interviews with site managers. The results are structured into two parts. Part 1: Horse-related tourism supply. This part includes sites and activities with tourism and leisure appeal, such as racecourses, competition venues, cultural and heritage sites, stud farms, private breeding or training facilities open to visitors or offering demonstrations, equestrian show venues, and sites hosting events such as fairs or exhibitions. Around 450 different activities have been identified as fully horse-related, including 214 activities related to culture, shows, festivals, fairs, and competitions, in addition to activities organized at the 231 racecourses. These activities generate around 5 million visits annually (1.7 million for culture/shows/festivals/fairs, 1.1 million for competitions, and 2.2 million for races) Ticket sale turnover is estimated at around €30 million for sites offering paid activities, including slightly less than €4 million for racecourses, for which ticket sales represent only a small share of total turnover. Part 2: Equestrian tourism in France. This part focuses on horseback tourism providers and tour operators. Around 1,000 FFE members hold an equestrian tourism label. Equestrian tourism constitutes the main activity for approximately half of them, that are of varying economic size, generating a combined turnover of €75 million and employing 1,450 staff. The other half mainly consists of riding schools offering diversified activities, in which equestrian tourism plays a more marginal role; their combined turnover of €135 million and 4,000 salaried jobs are therefore only partially linked to tourism. More than 3,300 riding tours organized by 750 certified clubs have been identified. In addition, around ten tour operators share the organized equestrian travel sector in France. The results highlight the economic importance of the sector and will help institutions better support these activities, their organization, and their impacts. The key figures that will be presented at the EAAP 2026 conference will also be used in regional exchanges with the equine sector, local authorities, and institutions, as well as in national economic publications and for international comparisons. Further work may be conducted at regional level (2026–2027), including methodological recommendations and additional field surveys to address remaining data gaps.

Relational practices in beef suckler systems: A literature review, holistic modelling, and emerging research questions

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Beef suckler systems require farmers to coordinate long- and short-term tasks under variable climatic and organisational constraints. This complexity shapes work organisation and the human–animal relationship. Across animal science, management science, and rural sociology, farming practices are seen as a decision-making process in which farmers carry out coherent tasks based on their goals and resources. Within this framework, some farmers develop relational practices: intentional actions aimed at fostering positive interactions with cattle and improving working conditions. These practices are grounded in farmers' representations of working with animals and contribute to safety, efficiency, and work satisfaction. Yet empirical studies on relational practices in beef suckler systems remain scarce and fragmented. This review aims to synthesise existing knowledge on relational practices in beef suckler systems, integrate these insights into a holistic model of the human–cattle relationship, and identify key research gaps and questions for future work. We combined a review of interdisciplinary literature with a conceptual modelling approach integrating human, animal, and environmental factors, work organisation, and interactional dynamics. On this basis, four published on-farm surveys from France, Spain, and Canada were analysed to identify convergent and divergent patterns in farmers' representations and practices. These studies covered diverse contexts, including differences in seasonal housing, geographical environments, breeds, herd sizes, and production conditions ranging from intensive to extensive or rangeland systems. Survey results align with many elements of our holistic model. Farmers view cattle as sensitive beings that react fearfully to aversive stimuli. Their main objective is to keep animals calm during handling, which improves both safety and efficiency. Improved animal welfare is sometimes explicitly mentioned. Reported relational strategies include culling difficult animals, accustoming cattle to human contact when time allows, and relying on calm, consistent behaviour supported by adequate handling facilities. European farmers emphasise regular contact, early-life interactions, and temperament-based culling, although positive tactile interactions are less frequently mentioned. The Spanish study highlights that farmers consistently identify cattle responses to unfamiliar people as problematic. Substantial variation exists across stock people and contexts, shaping both the feasibility and perceived value of relational practices. These differences raise questions about whether farmers adopt strategic or opportunistic approaches when building relationships with cattle. The literature and surveys show that relational practices can be a key component of work organisation in beef suckler systems, but evidence remains limited. Farmers rarely describe animals as active agents in the relationship, even though some acknowledge having favourite individuals. The model developed here highlights the need for additional surveys in diverse contexts to understand how relational practices emerge, how they interact with system constraints and other farming tasks, to what extent they are recognised as genuine work activities, and how they can be designed most effectively for both farmers and animals. Such insights are essential for developing interventions, training programmes, and breeding strategies that support safer, more effective, and mutually adaptive human–cattle relationships.

Traffic noise exposure alters horse behaviour at pasture

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Horses are pastured to meet behavioural needs for movement. However, for various reasons, pastures are often located close to motorways or busy roads, which exposes horses to constant traffic noise. In humans, pronounced and constant traffic noise is a critical factor that promotes health risks, such as cardiovascular diseases and auditory impairments. Given the horse's evolutionary adaptation as a vigilant and noise-sensitive prey species, chronic anthropogenic noise may lead to similar health issues and, further, interfere with natural behaviour and stress regulation. We investigated how pastured horses are affected by continuous traffic noise and whether habituation to the noise exposure can occur. With portable loudspeakers, we exposed twenty noise-naïve horses from three facilities (four social groups) to controlled motorway playbacks. After an initial habituation day, we collected baseline behavioural data on a pre-exposure day. Then, horses were exposed to traffic noise from 54 to 71 dB, simulating distances of 500 to 10 m to a 3-lane motorway, across two exposure phases, intersected by an intermediate wash-out day. Exposure durations ranged from 4 to 11 hours per day, depending on pasture schedules and local regulations. The order in which the six sound levels were applied was pseudorandomized on a day-to-day basis. After the second exposure phase, we collected data on a final post-exposure control day. Throughout the whole sampling period, we continuously measured the heart rate to monitor the physiological stress response. Behavioural observations quantified foraging, social, comfort, resting, alert, and flight behaviour. Full-reduced model comparisons of linear mixed models confirmed our hypotheses that constant traffic noise exposure alters horse behaviour at pasture and provokes a stress response. Horses reduced foraging time from 81% of the observation time at baseline conditions before traffic noise exposure to 41% during noise exposure ($\chi^2(7, N=216)=43.96$, $p<0.001$). They increased vigilance behaviour from a maximum of nine instances per observation period at baseline conditions to a maximum of 54 instances during noise exposure ($\chi^2(7, N=217)=71.78$, $p<0.001$). Partial behavioural habituation was observed across repeated exposures, with vigilance decreasing over time, though not returning fully to baseline levels (second exposure period: max. 25 instances). Mean heart rate increased during noise exposure (median: +9.97 bpm; $\chi^2(7, N=35320)=1065.07$, $p<0.001$) but remained elevated during wash-out and post-exposure periods, suggesting anticipatory or avoidance-related arousal. Despite signs of behavioural habituation, constant traffic noise affected both activity budgets and physiological state. Importantly, the horses increased the distance to the sound source specifically during louder noise levels ($\chi^2(6, N =217)=18.97$, $p<0.001$), thus decreasing the actually experienced sound pressure. This avoidance behaviour may represent an active coping strategy, mitigating perceived threat. These first findings highlight that exposure to constant traffic noise can compromise welfare in pasture systems and underscore the importance of structural mitigation measures, such as noise barriers, to safeguard horse welfare.

Acoustic relationships between bovine vocalizations and traditional herding songs: implications for human–animal communication and cattle handling under grazing conditions

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Communication between humans and animals plays a critical role in cattle management, particularly under grazing systems where auditory signals are commonly used to guide animal movement and behavior. Traditional herding songs represent culturally transmitted vocal signals used by farmers to interact with cattle; however, their acoustic relationship with bovine vocalizations under grazing conditions remains poorly understood. This study aimed to compare the acoustic characteristics of bovine vocalizations and traditional herding songs to identify sound patterns that may facilitate human–animal communication during grazing management. A descriptive comparative bioacoustic analysis was conducted using recordings of bovine vocalizations from Bos taurus and Bos indicus cows (n = 50) and traditional herding songs (n = 80) obtained from open-access sources and field recordings. Acoustic parameters were extracted using bioacoustic analysis and Music Information Retrieval (MIR) techniques, including spectral centroid, amplitude, dissonance, zero-crossing rate, and high-frequency content. Differences between vocalizations and songs were evaluated using ANOVA with Bonferroni post-hoc tests (statistical significance set at $P < 0.05$). Multivariate relationships among acoustic features were explored using principal component analysis (PCA). Herding songs exhibited longer durations (e.g., 4.2 ± 1.1 s vs. 1.3 ± 0.5 s; $P < 0.001$) and higher dominant frequencies (>2500 Hz; $\eta^2 = 0.42$; e.g., 2700 ± 350 Hz vs. 1450 ± 210 Hz; $P < 0.001$) with frequencies approximately 85% higher than those observed in bovine vocalizations, whereas bovine vocalizations showed shorter durations and frequencies ranging between 1115 and 1797 Hz. Multivariate analyses identified two distinct acoustic clusters separating bovine vocalizations from herding songs, with songs showing greater spectral variability and a higher proportion of high-frequency components (spectral centroid and high-frequency content, $P < 0.01$). These findings highlight structural differences between human vocal signals and bovine vocalizations while revealing acoustic characteristics that may influence cattle perception of human-generated sounds. The first two principal components explained 63.4% of the total variance. Understanding these relationships provides a quantitative framework for exploring how auditory stimuli used by farmers may affect cattle behavior and communication during grazing management. In addition, the acoustic patterns identified in both bovine vocalizations and herding songs provide a scientific basis for the development of species-adapted auditory stimuli. Such stimuli could be used as environmental enrichment tools to modulate animal behavior and improve welfare in grazing systems and holding areas, including pre-milking waiting pens or handling facilities.
Keywords: human–animal interaction; bioacoustics; herding songs; cattle communication; acoustic signals.

Behavioural indicators of the emotional state of outdoor managed pigs during an animal-human approach test

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Evaluating behavioural responses during a human–animal approach test provides insight into the animal's underlying emotional state when confronted with this potentially ambiguous social stimulus. To investigate this, we carried out an Animal-Human Approach Test (AHAT) with an unfamiliar human on 181 pigs in 16 outdoor pig farms from February to May 2023. The AHAT was performed on female, castrated and uncastrated pigs, aged from 3 months to 6 years (~10 pure/mixed breeds across all farms). The test consisted of 3 phases: the 'pig approach' phase (the animal had 30 seconds to approach an unfamiliar human located 2m away); the 'human approach' phase (the human approached the pig slowly if there was no previous contact); the 'scratch phase' (the human attempted to scratch the pig's forehead if there was contact). Scores were applied from 0 (most avoidant, no contact) to 3 (least avoidant, scratch accepted). The tests were video recorded to enable analysis of the following behaviours: Tail position (not recorded when the tail was docked, n = 8), ear position, vocalisation, and locomotion behaviours related to avoidance (run away, turn away, walking backward). Tail position was categorised as up, horizontal or down, curly or straight, loose or wagging. Tucked tails were never observed. Ears could be forward, neutral, backward or asymmetrical. Grunts and screams were also counted. Based on these observations the frequencies of the position of each body area, and of the changes in position were calculated. Analyses were carried out using RStudio (2022.07.02 version). General linear mixed models (glmmTMB package) and type II Anova (car package) were used to investigate the relationship between the AHAT score and each of the behaviours recorded during the test. In general, the frequency of changes in body area position increased with the AHAT score (i.e. there were more changes in less avoidant pigs; $p<0.01$ for each behaviour). The frequency of the tail being up was lower for pigs scoring 3 than 2 ($p=0.035$), which could indicate that more avoidant pigs were more alert. A curled tail (often interpreted as a sign of a positive emotional state) was less frequently observed when pigs scored 1, compared with pigs scoring 2 ($p=0.02$) or 3 ($p=0.06$). A loose tail was more frequently observed in pigs scoring 1 than 0 ($p=0.011$) or 2 ($p=0.009$). The frequency of ears being in a neutral position, which could indicate they were not actively orienting towards a sound, was higher for pigs with a score of 3 than a score of 0 ($p=0.026$). Screams occurred rarely, but more often in pigs with a score of 1 than 0 ($p<0.001$), 2 ($p=0.02$) or 3 ($p=0.001$). The frequency of grunts was higher for pigs with a score of 3 than a score of 0 ($p=0.011$) or 2 ($p=0.026$). The frequency of avoidance locomotion decreased with the AHAT score ($P < 0.05$ for all). These results confirm that body posture, vocalisations, and locomotion behaviours can provide insight into pigs' emotional states during human–animal interactions in outdoor farming systems. Overall, pigs that were less avoidant of humans performed fewer behaviours that are indicative of a state of alertness.

Conflict behaviours and potential "positive" behaviours in horses ridden in a low level training situation in their home setting

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Recently, a number of studies have highlighted the high occurrence of conflict behaviours (e.g., mouth opening, tail swishing) in competition settings in equestrian sports. This study aimed to evaluate the occurrence of conflict behaviours in horses in a low-level training situation (dressage test of 10 min in all three gaits including circles and volts on both hands) in their home setting. This study further aimed to explore whether conflict behaviour was negatively associated with behaviours proposed to indicate a potential "positive" state in riding horses from the literature (ears pointing forward, snorting, low head-neck position). A convenience sample of 31 horses of different types (Wambloods, Trotters, PRE, Danish Sport Ponies, Welsh Ponies and Mixes), sex (17 geldings, 13 mares, 1 stallion) and age (ranging from 4 to 29 years) took part in the study. The riding sequences were filmed and behaviour observed based on a predefined ethogram (counts of each behaviour). State behaviours (ear positions and head-neck position) were recorded in bouts of 4 sec, i.e., the position should be present for at least 4 sec to be counted, and if the position remained for more than 4 sec, another bout was counted. Effects of sex, age, environment (indoor or outdoor) and gaits on behaviour were analysed using generalised linear mixed models and associations between behaviours using the Spearman rank correlation (ρ). Horses exhibited an average of 5.2 conflict behaviours per minute with open mouth (45.3%) and tail swishing (23.2%) accounting for the majority, aligning with previous findings. Conflict behaviour was not affected by sex, gait or environment. Age, however, was associated with a significant reduction in conflict behaviour ($P=0.01$), with an estimated 4% decrease per year. Horses exhibited an average of 1.5 potential "positive" behaviours per minute with ears forward being the most frequently observed, accounting for 71.3% of the total "positive" behaviour. Potential "positive" behaviours were significantly less frequent in canter compared to walk ($P<0.001$) and in trot ($P=0.04$), with no significant difference between walk and trot ($P=0.14$). Additionally, horses ridden outdoors showed significantly higher rate of "positive" behaviours compared to those ridden indoors, whereas there was no effect of sex or age. Total conflict behaviour was significantly ($P<0.05$) moderately negatively correlated with total "positive" behaviours ($\rho=-0.43$), indicating that horses displaying more positive behaviours tended to show fewer conflict behaviours. In interpretation of the single indicators, it is particularly noteworthy that snorts were often observed directly after a conflict behaviour which might indicate that they are rather seen as a release of tension, i.e. after a conflict occurred than a purely "positive" indicator. Likewise, for example ears forward and total ear movement depended on the context. Ridden behind the vertical was only observed in very low frequency in the present study and ridden above the vertical in the present study setting referred more to a loose frame than a conflict behaviour which should be borne in mind in the interpretation and underlines the effect of the study setting. Overall, the present study highlights the complexity of interpreting behavioural indicators of equine welfare in different settings. A combination of different indicators is likely preferable along with taking into account the specific settings.

Human perception of horses' facial expressions: implications for equine welfare assessment

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Accurate recognition of emotional states is essential for assessing horse welfare. Facial expressions are increasingly used as non-invasive indicators of animal emotions; however, the ability of humans to correctly interpret these expressions remains poorly understood. Emotional processing involves several brain structures, and facial muscle activation modulates observable expressions according to the internal emotional state. Humans often rely on facial expressions to assess animal welfare; however, misinterpretation may compromise welfare assessment. This study aimed to evaluate how accurately humans perceive horses' emotional states from facial expressions. An online survey based on 20 photographs of horses representing different emotional systems according to Panksepp's emotional framework (discomfort, fear, sadness, anger, pleasure, relaxation and curiosity) was conducted. Images were previously coded using EquiFACS to objectively identify facial action units associated with each emotional state. Participants were asked to identify the emotion displayed in each image and to provide information about their demographic characteristics, experience with horses and frequency of contact, in order to assess whether these factors influenced their ability to correctly interpret the expressions. A total of 250 individuals with diverse backgrounds participated in the survey, including equine professionals (e.g. veterinarians, caretakers, riders and breeders) as well as individuals with no prior experience in analysing facial expressions. The results indicate that some emotions, such as curiosity, were more easily recognised (58% correct responses), whereas frequent confusion occurred between other emotional states, including those of opposite valence. Several participant characteristics influenced recognition accuracy, including age, gender, professional background, prior experience with horses, frequency of observing horses' facial expressions and frequency of contact. Having a profession related to the equestrian sector (58% had equestrian-related professions, of whom 23% were veterinarians) and frequent contact with horses (72% of participants) significantly improved response accuracy ($p < 0.001$), although an overall limitation in emotion interpretation was observed. These findings highlight the limitations of relying solely on human perception to assess equine emotions and emphasise the need to develop more objective and standardised tools to complement human observation and improve the assessment of horse welfare.

Perceptions of equine welfare and the human–horse relationship within the equestrian community

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Understanding how people involved with horses perceive equine welfare is essential for improving management practices and strengthening the human–horse relationship. This study analysed perceptions of equine welfare and daily interactions with horses among members of the equestrian community. An online self-report questionnaire was distributed between February and April 2025. The survey included 64 questions (closed questions, Likert-scale items and one open question) addressing perceptions of equine welfare, management practices and human–horse interactions. A total of 189 participants completed the survey, including horse owners, professionals and enthusiasts. Half of the respondents did not work in the equestrian sector, while the most represented professional groups were trainers (17%) and veterinarians (14%). Participants evaluated the importance of the five domains of animal welfare (nutrition, physical environment, health, behavioural interactions and mental state), and demographic variables such as gender, age, professional background and level of knowledge were analysed. Most participants (92%) considered horses to be companion animals or members of the family. Overall, aspects related to the absence of suffering—such as health, nutrition and adequate housing—were rated as highly important. In contrast, welfare dimensions associated with the presence of positive emotional states, including behavioural interactions and agency, were given comparatively lower priority. This suggests that horses' mental and emotional needs are not perceived as equally important as physiological or environmental needs. Gender emerged as the variable with the most marked differences (72% of respondents were women), with women tending to attribute greater importance to both physical and emotional aspects of welfare and reporting more empathetic and respectful practices. Younger participants, individuals not working in the equestrian sector, and those who considered horses as companion animals also tended to prioritise respectful interactions. Regarding the human–horse relationship, 63% of respondents reported that horses frequently initiate interactions with people (e.g., approaching during haltering or seeking tactile contact), and 71% reported regularly engaging in non-riding activities with their horses. Despite generally positive perceptions, respondents identified several barriers preventing welfare recommendations from being fully implemented in practice, including economic constraints, lack of owner knowledge, traditional management systems in equestrian facilities and logistical limitations such as space availability. These findings highlight a gap between recognising the importance of preventing suffering and promoting positive welfare states. Strengthening education and training initiatives that integrate emotional and cognitive aspects of equine welfare may help foster more comprehensive welfare practices and more positive human–horse relationships.

Young EAAP session - impact, career building and workshops

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The Young EAAP session brings impactful topics and speakers to the early career people at EAAP. This year's session consists of 3 parts. In the first part, we handle how we can prepare ourselves and our CV for life after PhD. We will discuss with experienced people in a variety of roles what skills they needed and missed in their current careers. The second part of the session, we will discuss impact in all its facets. We will deal with what types of impact there are (e.g. on the sector/farmer, on people, on an academic audience, etc.), and how we can fit it into our work. Topics that will pass by are e.g. science communication, industry collaborations, etc. As many funding agencies also require to specify "impact" in a research proposal, we will discuss how we can address this during funding acquisition. In the final part of the session, we organize a series of workshops around a variety of topic, including artificial intelligence, large language models, animal welfare, agile working in science, reproducible science and code, talking about failure, how to sell sustainable animal production, leadership and work/life balance. In each, we will interactively and in small groups work around these topics together with other young scientists. Everyone is welcome to join and participate.

Productivity and behavioural monitoring of dairy goats under heat stress conditions

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Heat stress compromises dairy goat metabolic homeostasis, behavior and overall productivity. Modernization and production demands require shifting farmer-observation management toward technological approaches. However, digitalization poses challenges, including monitoring in high-density housing and developing robust algorithms that run on devices with limited battery life. Continuous monitoring is particularly critical under heat stress conditions, which severely impact animal welfare and productivity. This study aimed to validate an accelerometer-based behavioral monitoring system and apply it to identify activity patterns affecting milk production under heat stress. Following algorithm validation, a field study was conducted from June to October 2024. Fifty activity collars running the developed algorithm were deployed on multiparous dairy goats. Activity patterns were derived from the labelled behavioral states, specifically “Eating”, “Moving”, “Lying”, “Inactive”, and “Others”, and were recorded as a percentage of activity at an 11-minute temporal resolution. To quantify environmental stressors, meteorological data was retrieved from weather station installed in the farm. Furthermore, intrinsic individual traits, specifically the number of kids at birth and the genetic value of each animal, were collected as baseline predictive features. Intrinsic biological factors established the primary baseline for milk yield, with the animal’s Genetic Value emerging as the dominant predictor, followed by the number of kids at birth. This indicates that individual baseline potential is the strongest anchor for the predictive model. Environmental conditions showed to be a dynamic factor modulating this baseline. Notably, the THI recorded during the night ranked as the second most important feature overall. Furthermore, its historical lags dominated the top tier of predictors, suggesting that the physiological toll of poor nighttime recovery is a far more critical determinant of next-day production than daytime heat peaks. Random Forest analysis demonstrated that while intrinsic biological traits, specifically genetic value, are the primary drivers of milk yield, environmental conditions act as critical dynamic modulators. Notably, nighttime THI and its historical lags were stronger predictors than daytime heat peaks, emphasizing that inadequate nighttime physiological recovery severely impairs subsequent production. Although accelerometer-derived behavioral metrics provided useful predictive fine-tuning, they remained secondary to genetic and thermal factors. Ultimately, the model reliably tracked herd productivity trends, highlighting the potential of wearable sensors and machine learning to optimize dairy goat management under heat stress.

Feeding preferences of sheep grazing dryland pastures in Montado: influence of crude protein and neutral detergent fiber

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Selective grazing behaviour in sheep not only affects animal performance but also has implications for animal welfare. Nutritional adequacy and access to preferred forage influence physiological and behavioural well-being. This study evaluated how crude protein (CP) and neutral detergent fiber (NDF) affect feeding preferences of sheep grazing Montado pastures under unrestricted biomass availability. The study was conducted at the ECO-SPAA experimental field, located at Herdade da Mitra (University of Évora, Portugal). A 4-ha area was subdivided into four experimental paddocks subjected to different treatments (with or without dolomitic limestone application × two stocking rates: 1 LU/ha and 2.5 LU/ha). In each paddock, 12 sampling points representing the existing botanical communities were previously identified, georeferenced, and physically marked. Field observations were carried out during four periods in 2024 (P1 – 20 March, P2 – 18 April, P3 – 13 May, and P4 – 20 June). On these dates, grazing locations of the animals were georeferenced and recorded by trained observers using binoculars at 10-minute intervals from sunrise to sunset. On the day preceding each observation period, pasture height was measured and samples were collected for laboratory determination of CP and NDF concentrations. Results showed that during P1 (CP > 14% at all sampling points) and P4 (CP < 10% and NDF > 59% at all sampling points), CP and NDF concentrations did not appear to influence grazing preferences, although selective grazing behaviour was observed across all treatments. However, during intermediate observation dates (P2 and P3), when CP showed high spatial variability (approximately 8–20%) and NDF variability was relatively low (45–56%), sheep exhibited selective grazing, preferentially grazing areas with higher CP and lower NDF concentrations. These results indicate that: (i) under conditions of high biomass availability, selective grazing occurred in all treatments, even at higher stocking rates; (ii) sheep preferentially selected areas with higher CP when spatial variability in CP was high within paddocks; and (iii) NDF concentration did not appear to significantly influence grazing preferences. These insights can guide pasture management strategies that promote both performance and animal well-being. Funding: This work is funded by National Funds through FCT – Foundation for Science and Technology under the Project UID/05183/2025.

Managing genetics of resistance to parasites in goats: Reviews at different scales of diversity to implement agroecological breeding systems in the tropics

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In tropical and subtropical regions, agricultural economies are strongly supported by goat production. Ensuring the sustainability of farming households' incomes in such low-input systems requires achieving a balance between the environment and the animals. Livestock farming practices now emphasize the genetic adaptation of animals to their environment rather than environmental modification. In this context, gastrointestinal nematode (GIN) infections represent a major limitation to goat production in the tropics. The agroecological approach is to make use of the genetic variation that allows some goats to cope better with infection although this genetic resistance of goat to GIN is sometimes debated. We conducted Systematic reviews of exploitable genetic diversity at different levels, including the genome, the population, and the breeding system. Following PRISMA guidelines, we searched Web of Science, PubMed and Scopus (December 2025) and selected studies that either (1) identified genomic regions linked to resistance, (2) estimated genetic parameters for resistance or resilience traits and (3) studies conducted on ruminants that assessed GIN infection in multi-species or mixed grazing system, and reported the level of system resistance/resilience within agroecological, low-input, or locally adapted production systems. At the genomic scale, data were extracted from 26 selected studies including 29 breeds across nine countries, and identifying 52 candidate genes or genomic regions associated with resistance traits, including fecal egg count, packed cell volume, and immunoglobulin levels. At the population scale, 38 studies were included representing 30 breeds distributed across tropical, humid subtropical, temperate and hot semi-arid climates zones. At the system scale we reviewed x studies that examine how differences in GIN resistance between ruminant species can be used to design more resilient agroecological grazing systems. Gene prioritization analysis using ToppGene identified 8 high-confidence candidate genes. Functional enrichment analysis revealed significant enrichment of the immune-related biological processes, leukocyte activation, cytokine signaling, pathogen recognition, and immune receptor activity. A comprehensive and quantitative meta-analysis of genetic parameters for resistance to GIN in goats revealed a global heritability estimate for resistance of 0.25 ± 0.06 from 18 studies including 20 breeds. A meta-regression analysis was performed in order to test the influences of latitude, age, infection type, statistical method, or maternal effects on genetic parameters. The first conclusion of our analysis is that resistance to GINs in goats has a relatively stable genetic basis, consistent with that obtained for sheep. The approaches at the herd scale include mixed-species grazing, adaptive management strategies and the integration of resistant breeds to strengthen system robustness. Our study provides consolidated knowledge to integrate genetic diversity in resistance to GIN in goats across multiple scales (gene, population, herd) in tropical environments. These constitute key levers for sustainably improving the productivity of low-input goat production systems facing parasitism.

Evaluation of Haemonchus contortus-derived extracellular vesicles in modulating sheep immune response in vitro

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Gastrointestinal helminths, particularly Haemonchus contortus, are major constraints to small ruminant health. Their survival is associated with excretory/secretory products, including extracellular vesicles (EVs), which act as key mediators in host-parasite communication. However, the interaction between H. contortus EVs and ovine innate immune cells remains poorly understood. This pilot study evaluated the ability of adult H. contortus EVs to modulate the in vitro immune response of sheep CD14+ monocytes. Adult parasites were recovered from the abomasum of sheep at slaughter. Following 24-h incubation in serum-free RPMI, EVs were isolated from the supernatant by sequential centrifugation, ultracentrifugation, and size-exclusion chromatography. Vesicles were characterized by nanoparticle tracking analysis and cryo-electron microscopy. CD14+ monocytes, isolated from ovine peripheral blood using Percoll gradients, were exposed to various EV concentrations. Assessment included MTT cytotoxicity assays, confocal microscopy for EV uptake, cytokine quantification (ELISA), gene abundance (qPCR), ROS production (cytochrome C assay), and apoptosis evaluation (Caspase 3/7). Data were analyzed using Shapiro-Wilk, ANOVA, or Kruskal–Wallis tests ($p<0.05$). Results showed that isolated EVs displayed typical EV-helminth morphology and were successfully internalized by monocytes. Exposure to non-cytotoxic concentrations induced measurable modulation of immune parameters. Significant variations in cytokine production, gene expression, ROS generation, and apoptosis were observed compared to controls. These findings indicate that H. contortus EVs interact with ovine monocytes to alter innate immune functions, providing a methodological basis for further mechanistic studies on parasite-driven immune modulation.

Genetic parameters for resistance to Small Ruminant Lentivirus (SRLV) in an experimental flock of dairy goats

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Lentiviral infections are responsible for chronic diseases that progress to severe clinical forms, such as arthritis in the case of caprine CAEV and lung disease in the case of ovine VISNA-MAEDI. There is currently no vaccine or cure for lentiviral infections, and eradication is impractical given their high prevalence. Literature from the last decade suggests that predisposition to lentiviral diseases in small ruminants has a genetic component. A major gene controlling seroprevalence has been identified in sheep. Knowledge of the genetic determinants of response to SRLV in goats is scarce. The objective of the present study was to estimate genetic parameters for SRLV-related phenotypes in a large-scale dairy goat herd. The data were collected from an INRAE experimental farm (P3R Bourges), where a herd of Alpine goats was observed over three years. Two complementary phenotypes of the goats' response to SRLV were measured once per year: i) seropositivity using a commercial ELISA test, and ii) presence of arthritis in the front carpal joints, reflecting the progression of the infection towards clinical signs (tolerance). To measure arthritis, we took circumference readings of the carpal and metacarpal bones using a tape measure. The Monicat score corresponds to the difference between the largest carpal bone circumference and the smallest metacarpal bone circumference. Arthritis is diagnosed if the Monicat score is 6 cm or greater (binary trait: presence/absence). Of a total of 1,106 individual measures from 578 goats, the seroprevalence was found to be 53.2%, and the prevalence of arthritis was found to be 34.6%. Both seroprevalence and arthritis increased with age, rising from 36% and 33%, respectively, in one-year-old goats to 94% and 59% in goats over four years old. Heritability was estimated by REML using a linear repeatability animal model with the wombat software. The pedigree comprised 1,841 animals. Heritability was equal to 0.10 (±0.06) for seroprevalence. Heritability for arthritis was 0.21 (±0.07) for the Monicat score and 0.13 (±0.05) for the binary outcome. The Pearson correlation of the estimated genetic values of seroprevalence with the two arthritis traits was then computed. The correlation ranged from 0.18 to 0.27, suggesting that the response to infection and the disease's clinical outcome (tolerance) are different genetic traits with different underlying mechanisms and genes. Taken together, these results suggest that there is a genetic component to the response to infection and the clinical form of SRLV, paving the way for future breeding control programmes.

Dairy goat welfare: Towards a welfare monitor with more animal-based welfare indicators

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The Dutch dairy goat sector is currently working on ways to improve the sustainability. In this context, an animal welfare monitor included as part of the Sustainable Goat Dairy Chain has been developed in the past years. In the light of recent developments and new insights in the field of animal welfare, the sector needed an evaluation of the existing animal welfare monitor and advice on further development in the direction of an animal welfare monitor with more animal-based welfare indicators. Thus the aim of this project was, with the existing animal welfare monitor as a starting point, to develop a new animal welfare monitor for dairy goats with the inclusion of more animal-based indicators. The 13 indicators of the existing monitor were mainly focused on stable design and management factors. Despite their relevance, they are not animal-related and therefore do not provide a factual insight into the welfare of the dairy goats on a specific farm. The inclusion of animal-based welfare indicators in the current monitor was therefore desirable. A literature study and an analysis of existing well-being monitors have resulted in a longlist of well-being indicators. The animal-based indicators of the longlist have been classified and assessed for validity, feasibility and reliability. This review against the literature formed the basis for an expert selection of a list of ten animal-related indicators, consisting of 1) hair and skin condition, 2) thermal comfort (prevention of heat stress), 3) thermal comfort (prevention of cold stress), 4) healthy udder, 5) eye/nose discharge, 6) clean hindquarters, 7) smooth gait, 8) intact skin of the head, limbs and body, 9) alert and social animals and 10) mortality in goat kids and adult goats. In addition to the indicators on the longlist, the ten selected animal-based indicators have been classified according to the six guiding principles for animal-worthy livestock farming, drawn up by the Council for Animal Affairs in the Netherlands. A protocol has been developed to test the selected animal-based welfare indicators. The draft protocol has been tested on five Dutch dairy goat farms. Based on the findings, the protocol was evaluated after each visit and adjusted in the interim where necessary. In the development of the protocol, thermal comfort, social animals, and mortality were not measured for practical reasons. The final protocol was tested at two farms to obtain data for testing repeatability between and within observers. Goats were assessed in 3 ways: in a separation pen, during walk along, or via pictures. The walk-along scores of all indicators were low (intraclass correlations for intra- and inter-observer < 0.40). For the separation pen and pictures the degree of repeatability between observers was qualitatively divided into the categories poor, minimal, fair and good across all statistics used (percentage agreement, PABAK, Cohen's Kappa, or Gwet's AC1). Wounds abnormal gait, nasal discharge and coat condition have been identified as well-being indicators with both a high estimated well-being impact and good repeatability. Abnormal gait is an indicator easily repeatable on the basis of a limited number of observations in which abnormal gait has been observed at the front legs (this could not be estimated for the back legs). Reliability scores of lameness, signs of inflammation, diarrhoea, presence of swelling, and itching could not be estimated because the prevalence was too low (<2.5%), however these indicators need further attention as they have a high impact on well-being.

Effects of a higher colostrum intake on health and growth of goat kids in their first 12 weeks after birth

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Respiratory problems are the most common health issue in goats, and particularly in goat kids in the first weeks of their life. Respiratory diseases can be caused by various infectious agents, but they depend also on management strategies, including provision of a sufficient quality and quantity of colostrum. This study investigated the effects of higher colostrum intake on goat kid health and growth in the first 12 weeks after birth. It was hypothesized that a higher colostrum intake in the first 24 hours after birth would lead to fewer clinical signs and better growth in goat kids compared to a standard colostrum intake. A total of 5 Dutch goat farms with kidding season in May-June and September were selected for the study. A total of 678 goat kids (average birth weight: 3.56 kg ± 0.02) originating from these farms were included in the experiment and they were assigned to one of these two treatments: 1) control group (CON): received an amount of colostrum of 15% of body weight (BW), and 2) experimental group (EXP): received an amount of colostrum of 22.5% of BW. In each farm, the CON and EXP groups were housed separately in two different compartments with similar climate and management. The first colostrum feeding was provided as soon as possible after birth (with a maximum of 3 hours after birth). The other feedings had to be fed within 24 hours at the latest. Clinical health assessment and BWs were measured at birth and in week 2, 4, 6, 8, 10 and 12. Use of medications and mortality were recorded. Clinical health data were analyzed as binary variables (0= normal and 1= with a clinical problem) with a generalized linear mixed model (GLIMMIX), whereas average daily gain and BWs were analysed with a linear mixed model. Both models included fixed effects of treatment (CON vs. EXP), farm (1 to 5) and their interaction. Treatment did not affect absolute BW and overall growth, but there was a significant treatment × farm interaction ($P<0.05$). The prevalence of clinical problems was overall low (3.2%), and the number of goat kids with nasal discharge during the whole experiment was lower in the CON group than in the EXP group ($P=0.01$). The number of animals treated three (5.1% vs. 2.4%) or more times (2.6% vs. 0.3%) was higher in the EXP group, but the number of group treatments was higher for the CON group than for the EXP group (6 vs. 2). The results of the current study did not match expectations and the hypothesis over the beneficial effects of feeding more colostrum was not proven, with the exception of one farm. This was likely an effect due to the inclusion of farms with above-average management and the kidding season (not in winter), which also might have contributed to a lower prevalence of health problems. Additional research is recommended, especially to determine the effects of a higher colostrum amount in the longer term.

Examining the relationship between somatic cell score, test day milk, and milk composition in different stages of lactation in Cyprus Damascus goats

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For several goat breeds, including Cyprus Damascus goats, the relevance of somatic cell count (SCC)-based selection and the establishment of specific threshold values to differentiate between healthy and pathological milk SCC have not been clearly defined. Therefore, the objective of this retrospective longitudinal study was to study SCC fluctuations during lactation and to examine the relationship between varying SCC levels, test day milk and milk composition at different stages of lactation in Cyprus Damascus goats. Data were obtained from the Cyprus Damascus goat research herd of the Agricultural Research Institute between November 2021 and March 2025 and included 513 lactations from 269 dams, with records collected during early (50-55 days), mid (81-86 days) and late (113-117 days) stages of lactation. For statistical analysis, the SCC distribution was normalized using a logarithmic transformation to somatic cell score (SCS). Mixed linear models were employed to analyze test-day milk and composition traits in relation to SCS levels, categorized into 6 classes based on their deviation from the mean. Separate analyses were conducted for primiparous and multiparous goats. Throughout lactation, multiparous goats exhibited significantly higher SCC and test-day milk than primiparous goats (mean SCC values of 1.48×10^6cells/mL in primiparous goats and 1.97×10^6cells/mL in multiparous goats ($p< 0.05$)). In primiparous goats, the mean SCC ranged from 1.29×10^6cells/ml in early lactation, 1.66×10^6cells/ml in mid-lactation, and 1.49×10^6cells/ml in late lactation, whereas in multiparous goats, mean SCC ranged from 2.27×10^6cells/ml, 1.57×10^6cells/ml, and 2.06×10^6cells/ml across the respective stages. In primiparous goats, test day milk ranged from 1.35 kg/day in early lactation, 1.34 kg/day in mid-lactation, and 1.24 kg/day in late lactation, whereas in multiparous goats, test day milk ranged from 2.12 kg/day, 1.80 kg/day, and 1.62 kg/day across the respective stages. SCS exhibited a significant negative correlation with test day milk in multiparous goats during both early ($r = -0.14$, $p< 0.01$) and mid-lactation ($r = -0.17$, $p< 0.001$), whereas no significant relationship was detected in primiparous goats. When comparing goats with low SCS (classes 1–2) to those with high SCS (classes 5–6), no significant relationship was observed in primiparous goats, while multiparous goats with elevated SCS produced significantly less milk compared to those with lower SCS, with daily milk losses reaching 0.46 kg/day in early lactation ($p<0.05$), 0.37 kg/day in mid-lactation ($p<0.05$), and 0.24 kg/day in late lactation. Similarly, SCS was negatively corelated with lactose concentration, with the relationship being significant throughout early ($r = -0.10$, $p< 0.05$), mid ($r = -0.25$, $p< 0.001$) and late lactation ($r = -0.26$, $p< 0.001$) in multiparous goats and only in mid-lactation in primiparous goats ($r = -0.19$, $p< 0.05$). Conversely, SCS was positively correlated with milk protein concentration, with significant associations observed in multiparous goats throughout early, mid, and late lactation ($r = 0.21–0.24$, $p< 0.001$) and only in late lactation in primiparous goats ($r = 0.20$, $p< 0.05$), indicating that higher SCS is associated with milk compositional changes, particularly in older goats.

Sheep Pox outbreaks in Bulgaria: Epidemiological spread and socio-economic impacts on the sheep farming sector

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In 2025, Bulgaria experienced a substantial wave of sheep pox outbreaks, posing serious challenges for disease control and the sustainability of the national sheep sector. The disease may have been introduced through neighboring Greece, where 1 539 outbreaks were reported during the same period. This study examines the epidemiological spread of sheep pox in Bulgaria and evaluates its socioeconomic consequences for the sheep and goat sector. Official data and field information from affected municipalities and regions for the period 01 January–31 December 2025 were used to assess both the geographical distribution of the disease and the impact of control measures on livestock production. Between 1 January and 31 December 2025, a total of 192 outbreaks were reported in 7 administrative regions, affecting 26 municipalities and 83 settlements in the country. According to the Animal Disease Information System (ADIS), 23 305 small ruminants were stamping out, including 21 968 sheep and 1 337 goats, as part of disease control measures. The Plovdiv region was the most severely affected, accounting for 143 outbreaks (75% of all cases). Public expenditures related to outbreak eradication, including veterinary interventions, carcass disposal and farmer compensation, reached approximately €15 million. Beyond these direct losses, significant economic and structural consequences were observed. Farmers experienced the loss of breeding animals, disruption of production cycles and reduced milk output in country, resulting in income losses and decreased farm viability. At sector level, field data indicate substantial reductions in valuable native sheep breeds, including population declines of approximately 30% in Patch-faced Maritza breed and 43% in White Maritza breed whose natural range is in the center of the sheep pox outbreak, increasing the risk of genetic erosion and potential breed extinction. Prolonged restrictions on the re-population of 192 affected holdings, lasting nearly one year—have further intensified these effects by limiting the recovery of production capacity of sheep farms and native sheep genetic resources, and by increasing the risk of sheep farm abandonment and transition to non-agricultural activities or beef cattle breeding. The management of the outbreaks also revealed competing policy priorities of the government, including the protection of export markets for sheep milk products and growing concerns regarding animal welfare and the preservation of sheep populations. In Bulgaria, control strategies were based on procedure of stamping-out and sheep and goat movement restrictions, without the use of vaccination, raising questions regarding the effectiveness of current control approaches under field conditions. Balancing effective sheep pox disease control with the long-term sustainability of the sheep sector therefore remains a key challenge for future livestock policy.

Cooperative handling strategies for group-housed sheep: Short-term training to improve welfare during routine procedures

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Sheep are typically held in large groups and are consequently managed less at the individual level. In contrast, smaller flocks more often permit individual-based handling. Nevertheless, essential husbandry procedures—health checks, clipping, and claw trimming—require individual restraint. As prey animals, sheep are highly susceptible to stress, particularly when isolated or physically fixated. Although their relatively small size and light weight allow manual restraint (requiring manpower), such procedures remain markedly stressful for the individual. We evaluated an alternative, cooperative handling approach for group-housed sheep, emphasizing resilience, motivation, attention capacity, and tolerance of human contact, essential for a research environment. Special consideration was given to species-typical behavioural characteristics, including intense group cohesion, easily triggered flight responses (initiated by the focal or conspecifics), variable individual flight distances, differences in boldness and motivation, threatening behaviour, and learned aversion or blocking. Two structured training protocols were implemented consecutively in a group of eight sheep over a two-week period using repetitive short sessions of approx. 8 min per animal (5–15 min). Before the training phase, the sheep had not been subjected to any behavioural training. However, they were routinely handled for health checks, incl. clipping and claw trimming, and pasture change, which were described as stressful conditions with high arousal and the necessity of several people for individual fixation. Thus, the first training aimed at sheep-induced voluntary contact through an advance-and-retreat paradigm combined with pleasant tactile stimulation (e.g., tickling) as positive reinforcement to counteract previously experienced negative interactions. The second training focused on haltering to allow loose fixation and leading. All training sessions were videotaped, and the progress was scored based on achievements (e.g., approach, touching the nose). Initially, all sheep showed high arousal, flight behaviour, and partly threatening behaviour, resulting from previous negative and limited experiences with humans. After the two weeks, all eight sheep were reliably caught in a self-motivated way and haltered within 10–60 seconds. Two individuals accepted haltering within the first 2 days (min. training time: 1 h), whereas the most sceptical individual required 13 days (max. training time: 3.75 h). Based on the observations, progress likely depended on individual expectations, prior experience, and the degree of bidirectional communication established during training. Essential for the sheep's improved tolerance were respecting their individual capacity for cooperation and reliable human behaviour. After the two weeks, the training effects generalized to other handlers, particularly in less sceptical and bolder individuals. All eight sheep displayed reduced arousal during routine health checks, despite necessary group trapping. In conclusion, cooperative care in group-housed sheep can be time-effectively integrated into daily routines using structured, low-intensity sessions. Its potential to reduce stress responses, facilitate handling, and decrease manpower, equipment use, and restrictive fixation techniques is now further investigated by implementing it into daily routines by the animal caretakers. Cooperative flock management represents a practical, welfare-oriented strategy. It provides a future perspective for refining key management practices in sheep production systems and in other environments where sheep need to be handled individually, such as livestock shows.

Impact of dietary supplements, breed and lactation stage on milk somatic cells count in sheep and goats

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Somatic cell count (SCC) is a key indicator of udder health in dairy small ruminants and strongly influences milk yield, quality, coagulation properties, and cheese yield. Previous studies have examined the effects of breed, lactation stage, and nutritional strategies, particularly dietary supplementation with oils or agro-industrial by-products, on SCC. However, results are not univocal, and a comprehensive comparison across studies is lacking. The aim of this study was therefore to evaluate, through a literature-based analysis, the impact of some factors as dietary supplementation, breed, and lactation stage on milk SCC in sheep and goats. A literature review was conducted using following keywords: somatic cell count, SCC, sheep and goat nutrition, milk quality, by-products, and oils. Review papers and studies lacking milk analysis were excluded, resulting in 32 eligible publications. Log-transformed SCC values (LogSCC) were extracted from 14 sheep and 18 goat studies published between 2006 and 2022. Data were analysed separately for sheep and goats using mixed models including lactation stage and supplementation type (oil or by-product) as cross-classified fixed effects and article as a random effect. A reduced model was also run for the three sheep breeds represented in more than one study (Sarda, Chios, Comisana; 10 articles), with breed added as a fixed effect. Supplementation type did not significantly affect LogSCC in any analysis. Lactation stage was significant only for sheep, with early lactation showing higher LogSCC than mid or late lactation. Among breeds, Chios sheep had the highest LogSCC (6.33 ± 0.20), followed by Comisana (4.32 ± 0.17) and Sarda (3.91 ± 0.12). In conclusion, dietary supplementation with oils or agro-industrial by-products does not appear to be an effective strategy for reducing SCC in sheep and goat milk. Greater attention is particularly required during early lactation, when the mammary gland is exposed to increased physiological stress due to the natural modulation of immune defence mechanisms associated with the abrupt transition to machine milking. If not properly managed and cleaned, machine milking may also act as a vehicle for pathogen transmission. Breed effects were detected only in sheep, suggesting that genetic background, production level, and farming system may contribute to differences in SCC. Acknowledgements: This work was funded by the Italian National Recovery and Resilience Plan (PNRR), Mission 4 “Education and Research”, Component 2 “From Research to Enterprise”, Investment 3.3 “Innovative PhD programmes addressing the innovation needs of enterprises and promoting the employment of researchers by companies” (Ministerial Decree No. 630/2024), and co-funded by CAO Formaggi, Sardinia.

CMT Scoring of ewe milk 15-70 days after lambing as an indicator for mastitis in commercial meat sheep flocks

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Statistics from the Norwegian Sheep Recording System (SRS) indicate mastitis as the main culling reason for Norwegian White sheep (NWS). An average case is costly and includes deaths of both ewe and lambs, reduced weight gain of lambs, replacement costs, extra work and treatment costs. It is thus very important for the industry to improve resistance against mastitis. High milk somatic cell counts in the milk are an immune response to a bacterial infection. The California Mastitis Test (CMT) is a relatively easy field test to use for the categorization of milk somatic cell levels. The test is frequently used in dairy cattle, but less frequently in meat sheep. The aim of the current study was to investigate if CMT scoring of ewe milk 15-70 days after lambing can be used as an indicator for mastitis. CMT was performed by mixing CMT liquid with milk in a small testing plate and categorizing the milk somatic cell level on a leveraged scale from 0 to 4 depending on the reaction between the milk and the CMT liquid. The typical production cycle of Norwegian meat sheep includes indoor lambing in early spring, grazing cultivated pasture around the farm from around 2-3 weeks of age of lambs, outfield pasture during summer, gathering of sheep from outfields in early September and grazing cultivated pasture around the farm during the fall. Recording flocks in the Norwegian breeding system are required to weigh lambs at birth, during spring (15 – 70 days of age), and at weaning (90 – 180 days of age) as well as to record the reason and date of death/culling of the ewe. Registrations should be stored in the SRS. As a part of a field study, 34 of the recording flocks were asked to check ewes for scars on the udder and teats, udder lumps, and to perform CMT scoring of the milk of the ewes 15 – 70 days after lambing and prior to release on outfield pasture. Udder lumps were scored in three categories: no lumps, lumps smaller than 4 cm, and lumps larger than 4 cm. Recordings should be done separately from both mammary glands and the highest score kept for further analyses. Average daily gain (ADG) of the litter from birth to spring weighing was computed as the sum of ADG over lambs divided by litter size. Both biological lambs and fostering lambs were included. In total, 1865 NWS ewes participated in the study. The risk ratio showed an almost 4- and 7-times larger risk, respectively, of being culled due to mastitis if the ewe had CMT-scores of 3 and 4, respectively, in at least one of the mammary glands at CMT scoring during the spring, indicating CMT scores 3 and 4 as possible early indicators of culling due to mastitis prior to the next lambing season. Also, a larger proportion of CMT scores of 3 and 4 were associated with udder lumps larger than 4 cm compared to lower CMT-scores. Least square means of average daily gain of the litter from birth to spring weighing corrected for herd, age at spring weighing, age of dam, litter size at spring weighing, and average gender of the litter show reduced ADG of lambs of higher CMT-scores, with a difference of 49 gram between CMT-score 0 and CMT-score 4. Despite the fact that field responses indicated challenges in milking the ewes, and in some cases in performing of the CMT scoring, we conclude that CMT score performed 15-70 days after lambing can be used to identify elevated milk somatic cell counts (mastitis), leading to reduced daily gain of lambs from birth to spring weighing as well as a larger risk of the ewe being culled due to mastitis prior to next lambing season.

Blood Transcriptome and Immune Cell Profiling Highlights Host Defence Variations in Naïve and Immune Goats infected with Gastrointestinal Nematodes

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A major economic and health concern in small ruminant production is gastrointestinal nematode (GIN) infection. The aim of this study was to compare the blood transcriptome of naïve and immune goats at different time points after Haemonchus contortus, as well as the abomasal mucosa and lymph node transcriptomes at late infection. Besides, identify the immune cell profiling variation between naïve and immune kids from transcriptome data, in order to identify different pathways and immune cell variation involved in the host defence. Naïve and immune Creole kids were orally infected with 10,000 H. contortus infective larvae (L3), and blood was sampled at 0, 3, 8,15, 21, 35 and 49 days post-infection (dpi). At 49 dpi, all the animals were slaughtered and abomasum and lymphe node tissues were collected. RNA-seq analysis showed that the highest number of different expressed genes (DEGs) was recorded when comparing immune versus naïve at 35 dpi, while the wider range for log2 fold change was recorded at 21 dpi. Results for canonical pathways showed that inhibition of matrix metalloproteases, leukocyte extravasation signalling, oncostatin M signalling and glioma invasiveness signalling pathways were significantly different between naïve and immune kids at early infection time in blood. Meanwhile, at 49 dpi, there was no identified different pathways when compare immune versus naïve kids at abomasum tissues level. In the other side, at lymph node level, several immune and cell healing pathways were significantly different between immune and naïve kids. Principal components analysis explained 74%, 66% and 71% of the variance in immune and naïve animals based on immune cell types identified from differentially expressed genes (using CIBERSORTx) at 49 dpi in blood, abomasum and lymph nodes tissues, respectively. Deconvoluted transcriptomic data showed that blood B cells were significantly higher in immune kids at different time points post infection compared with naïve kids. Meanwhile, CD4 and CD8 T cells proportions depend on the tissue examined and the time post-infection reflecting the development of the immune response. In conclusion immune cells proportion could serve as a reliable indicator of a developed immune response against GIN.

Bioclimatic profile of the rearing areas of local sheep breeds in Montenegro defined by multivariate analysis

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Montenegro possesses a significant diversity of autochthonous sheep breeds adapted to unique and contrasting geographical landscapes, ranging from Mediterranean coastlines to high alpine massifs. Understanding the bioclimatic specificities of these breeding areas is crucial for defining the adaptive capacities of local populations. Autochthonous sheep breeds in Montenegro represent a key component of national livestock biodiversity, developed through long-term adaptation to specific agro-ecological conditions. This study aimed to precisely define the bioclimatic profile of the rearing areas of seven local populations: Pivska pramenka, Bardoka, Zetska žuja, Sora, Sjenička ovca, Piperska žuja, and Ljaba, using multivariate statistical methods. Such characterization is essential for developing sustainable conservation strategies for animal genetic resources under ongoing climate change. The research covered 24 primary rearing locations across Montenegro. A historical climate dataset (1979–2018) including precipitation, minimum and maximum temperatures, wind speed, and altitude was utilized. Thirteen bioclimatic variables were derived, focusing on annual mean temperature (BIO1), annual precipitation (BIO12), and precipitation seasonality (BIO15). Data were processed using Principal Component Analysis (PCA) and Hierarchical Clustering on Principal Components (HCPC) to perform ecological classification of the areas and identify patterns of seasonal animal movement. The multivariate analysis revealed three distinct bioclimatic profiles corresponding to the major geographical and ecological zones of Montenegro. Cluster 1 (high-altitude) is characterized by rearing areas located at elevations above 1400 m, with low annual mean temperatures (BIO1 ≈ 5°C) and pronounced wind variability. Pivska pramenka and Sora are the predominant representatives of this climatic zone. While Pivska pramenka shows a high degree of year-round stationarity, Sora exhibits a mixed pattern, with part of the population remaining in the harsh conditions of Cluster 1 and another part moving seasonally towards more temperate zones. Cluster 2 (mid-altitude) comprises transitional areas at elevations between 800 and 1100 m, characterized by a moderate temperature regime. Sjenička ovca and Piperska žuja are consistently associated with this cluster, indicating predominantly sedentary management in climatically more stable environments without pronounced vertical migration. Cluster 3 (lowland–Mediterranean) includes areas with the highest temperatures (BIO1 > 15°C) and the lowest annual precipitation, but with the most pronounced precipitation seasonality (BIO15 > 50). Zetska žuja is well adapted to stationary production systems within this warm climatic zone. Results confirm that the bioclimatic profile of the rearing areas for breeds such as Bardoka and Ljaba changes seasonally due to vertical transhumance, where animals stay in the warmer Cluster 3 during winter and move to Cluster 1 or 2 during summer to avoid heat stress and utilize mountain pastures. Overall, the findings confirm a high degree of bioclimatic specialization among Montenegrin local sheep populations. The identified clusters indicate that traditional rearing systems, including transhumance, are key adaptive mechanisms to specific climatic regimes. These findings provide a scientific basis for long-term management and in-situ protection of indigenous populations in the context of increasing climate instability.

Within-day dynamics of milk components in intensively managed Saanen dairy goats.

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Understanding variation between morning (AM) and afternoon (PM) milk components is essential for accurate estimation of milk quality and composition. In dairy cattle, milk yield is typically greater in the AM while fat, protein and total solids are greater in the PM. Evidence in dairy goats is limited. Published data is based on small and regionally concentrated populations (12-40 goats) but suggests that milk yield and lactose may be greater in the AM, protein greater in the PM, and fat similar between AM and PM milkings. However, data from larger, intensive systems and from high-producing Saanen goats are lacking. We compared AM and PM milk components in high (HP) and low (LP) producing early lactation Saanen goats (n = 52) to quantify within-day differences and their interaction with production group. Individual milk samples (20mL) from HP (n = 28) or LP (n = 24) producing dairy goats were collected once in the PM and again the following AM for 7 weeks as part of a larger experiment. Samples were subject to milk component analysis using a Foss MilkScan Mars milk analyser (Foss Analytics, Hillerød, Denmark) that was specifically calibrated for goat milk to determine fat, protein, lactose, and total solids. Daily milk yield was recorded automatically using in-line electronic milk meters (MidiLine SG Parlor, DeLaval Inc., Australia). This was a retrospective experiment, and yield was automatically recorded daily rather than by AM or PM volumes. Thus, AM or PM yields were determined on only 5 occasions throughout the 7 weeks of sampling. Data was log10 transformed and analysed using REML in Genstat (22nd ed.), and presented means have been back-transformed and presented ± standard error of difference (SED). Milk yield was greater in the AM compared to the PM (2.0 L vs. 1.2 L; ±0.05; $P < 0.001$) on the 5 occasions this was compared. Overall, the HP goats had greater quantities of all components compared to the LP goats including fat (4.0% vs 3.5%; ±0.01; $P < 0.001$) and protein (3.3% vs 3.2%; ±0.001; $P = 0.008$). For both HP and LP goats, PM milk samples had greater proportions of fat (4.4% vs. 3.2%; ±0.001; $P < 0.001$), protein (3.3% vs. 3.2%; ±0.001; $P < 0.001$), lactose (4.7% vs 4.6%; ±0.001; $P < 0.001$), and total solids (12.8% vs. 11.5%; ±0.002; $P < 0.001$). These findings indicate clear and consistent differences between AM and PM milk quality and quantity. The PM milk was richer in fat, protein and total solids, and HP goats exceeded LP goats for all components. For the most accurate estimate of 24hr milk quality, averaging AM and PM component values or, if only one milking is used, standardising to AM or PM sampling is recommended. The findings partially agree with current literature, but lactose was greater in PM samples (a pattern commonly reported in dairy cattle), which may reflect differences in milking interval, feeding timing or circadian regulation of mammary metabolism. Variation can affect cheese yield estimates and payment systems based on fat and protein. Further work on the metabolic and lipidomic bases of variation and on economic consequences is warranted.

Farmer attitude to ovine lameness - Where do they seek information when a problem occurs?

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Ovine lameness impacts production efficiency, animal welfare and coupled with the associated treatment and preventative costs reduces farm profitability. Information on how to control and manage lameness is widely available but is sometimes conflicting or limited in its effectiveness. There is a paucity of information on where Irish sheep farmers go for information on ovine lameness treatment and control strategies. A survey of Irish sheep farmers on ovine lameness identification, control and treatment was conducted opportunistically and non-randomly between January 2023 and May 2024. Online respondents were gathered through promotion on Teagasc social media platforms. In-person respondents were collected at livestock sales and attendance at Teagasc sheep events with responses subsequently collated via 'Survey Monkey'. Following collation a combination of SAS Version 9.4 and R studio 4.5 were used for data screening and analysis. Results from this survey showed farmer reported median ovine flock-level lameness prevalence of 6.0%, with 47.0% of farms reporting that Contagious Ovine Digital Dermatitis (CODD) was present in their flock. As past of this survey participants were asked where they sourced information on ovine lameness. The most frequently chosen source of information on lameness was 'My Vet', reported by 30.0% (n = 92 [95% CI 25.0–35.5]) of farmers, followed by 'Own knowledge' (n = 38 [12.3%, 95% CI 8.8–15.8]), a combination of 'Farming media and veterinary advice' (n = 35 [11.4%, 95% CI 8.2–15.6]) and 'Farming media' (n = 24 [7.8%, 95% CI 5.2–11.6]), while other combinations such as advisors, farm knowledge transfer events, or other farmers were reported by smaller proportions respectfully. These results, particularly the high percentage that rely on 'Own knowledge' for the control and treatment of ovine lameness demonstrates the need for having consistent and effective KT messages presented to farmers and industry personnel in order to reduce the impact of lameness on production and welfare.

Substrate pH: A Critically Overlooked Microenvironmental Factor in Black Soldier Fly Larvae Performance and Systematic Reporting

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pH is a fundamental physicochemical parameter that affects microbial community structure, enzyme activity, and nutrient solubility. In black soldier fly (Hermetia illucens) (BSF) production, the substrate forms both the primary nutritional source and physical habitat for the larvae. Despite this biological relevance, quantitative relationships between pH and larval performance are poorly understood because pH is inconsistently reported. This exploratory study utilized a multi-study dataset (N = 635 observations) to highlight the importance of pH and advocate more systematic reporting. Analysis revealed reporting is exceptionally sparse: initial pH (Initial_pH) was recorded for only 15.3% of observations (n = 97), and final pH (End_pH) for only 10.2% (n = 65), limiting the paired subset to 65 observations. For these, the average initial pH was 5.67 ± 1.13 (range 3.61–8.40) and the average end pH was 7.25 ± 1.35 (range 4.03–9.11), with a mean change (Delta_pH) of 1.58 units (range −1.90 to 4.82). The Pearson correlation between Initial and End pH was weak ($r = 0.211$, $P = 0.092$), suggesting that other factors, potentially such as larval activity (feeding, excretion, bioturbation) and microbial processes, influence the chemical environment, diluting the impact of initial substrate conditions. Four modelling specifications, including ordinary least squares (OLS) and mixed-effects models (REML estimation) with study as a random intercept, were used to evaluate associations. Single-predictor OLS models demonstrated a negative association between Initial pH and performance outcomes; larval growth rate decreased by 3.14 ± 0.54 mg/d ($R^2 = 0.370$, $P < 0.0001$) and larval final wet mass decreased by 22.82 ± 4.32 mg ($R^2 = 0.279$, $P < 0.0001$) per unit increase in pH. In contrast, End pH associations were weaker and outcome-specific ($R^2 = 0.105$, $P = 0.034$ for larval growth rate; $R^2 = 0.012$, $P = 0.420$ for larval final wet mass). Accounting for study-level clustering in mixed models attenuated these coefficients but confirmed the negative relationship of Initial pH with larval growth rate ($\beta = -2.26 \pm 0.63$, $P = 0.0007$) and larval final wet mass ($\beta = -15.67 \pm 4.97$, $P = 0.0024$). In these models, End pH was no longer a significant predictor of larval final wet mass ($P = 0.267$). Neither the interaction between Initial and End pH nor the absolute change in pH (Delta_pH) meaningfully explained variance ($P > 0.15$). The analysis also revealed high intraclass correlation coefficients (ICC), ranging from 0.448 to 0.591, indicating that 45–59% of total variance was attributable to between-study heterogeneity, likely reflecting differences in substrate composition, larval density, and climate. While this dataset is observational and limited in size, precluding definitive causal inference, the persistent negative association between initial pH and performance supports the hypothesis that substrate pH may influence larval performance. Controlled experimental studies are needed to determine whether moderate acidification improves larval growth rate. Further studies should examine whether changes in microbial activity or nutrient accessibility explain any growth response to pH manipulation. This study serves as a vital call to the BSFL research community to systematically monitor and report initial and end substrate pH to support future optimization of rearing conditions and determine relevant interactions.

Bugs in the System: Have the economic and environmental benefits of insect farming been overstated?

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Insect farming has attracted over $1.5 billion in investment in Europe, with the sector positioned as a promising route to reducing dependence on fishmeal and soy in animal feed. Yet several leading companies have gone bankrupt, and investment fell sharply between 2022 and 2026. This presentation examines whether the assumptions underlying the research literature hold up against commercial realities. The scientific literature has broadly supported the environmental and economic case for insect farming. However, much of this evidence appears to rest on fragile foundations. Most life cycle assessments have been conducted at small or pilot scale, often in tropical or semi-tropical settings whose conditions may not be transferable to Western industrial production. Cost projections have relied on theoretical parameters that diverge from actual farm operations, and a pervasive assumption persists that insect farms will utilise food waste at an industrial scale, despite persistent regulatory, logistical, and safety barriers. These gaps between published research and commercial realities may have contributed to overly optimistic expectations among investors, policymakers, and feed industry stakeholders. Based on a series of peer-reviewed studies by the author, this presentation provides a comprehensive assessment of the challenges facing the insect farming sector. When more realistic inputs are used, the conclusions drawn from the literature appear to change substantially. In the feed market, insect meal appears to generate higher greenhouse gas emissions than soybean meal and fishmeal in most scenarios relevant to Western production, and the circular economy narrative central to the sector's value proposition remains largely aspirational at this stage. The economic outlook appears equally challenging: insect meal currently costs several times more than conventional alternatives, a gap that available modelling suggests is unlikely to close under realistic near-term conditions. In the human food market, these environmental and economic limitations are compounded by market dynamics, as insect-based products appear to overwhelmingly displace plant-based foods with already low environmental footprints rather than meat, thereby further undermining the sustainability case for entomophagy as a dietary shift strategy. These findings may be directly relevant to stakeholders in the feed industry and policymakers. They help identify the critical barriers that would need to be addressed for insect farming to deliver on its promises, while also flagging strategies that appear unlikely to succeed. While niche applications may still offer genuine benefits, for the sector to build a credible future at scale, research and investment decisions should be grounded in evidence that reflects actual industrial conditions rather than theoretical projections. Bridging the gap between theoretical promises and industrial realities will require a collective effort from researchers, industry actors, and policymakers to produce and rely on data that accurately reflects the conditions under which insect farming operates today.

Eclosion and mating postponement, how it influences oviposition in black soldier fly (Hermetia illucens)

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Egg production is a key indicator in black soldier fly (Hermetia illucens, BSF) breeding systems, as it determines both the supply of progeny and the proportion of larvae that can be allocated to biomass production. For commercial breeders, the ability to manipulate the insect's life cycle is valuable for aligning emergence, mating, and oviposition with operational schedules. Two potential interventions are cooling pupae to delay development and keeping adults in darkness to postpone mating and subsequent egg laying. However, these measures have biological limits: excessive cooling reduces emergence and egg output, and prolonged darkness may disrupt mating success. Earlier work showed that long-term cold storage (>10 days at 15 °C) is detrimental, yet unexpectedly, short-term cooling (2 days) increased egg production by 28% compared with non-cooled pupae. This raised the question of whether brief cold exposure during the pupal stage could reliably enhance egg production. In addition, we examined whether delaying mating by storing newly emerged flies in darkness affects total egg output. To address these questions, four trials were performed. Each trial consisted of 24 reproduction cages (60 × 60 × 90 cm), each containing a single batch of 250 g of pupae (≈2000 flies), maintained under uniform conditions (30 °C, 80% RH) with controlled lighting, water access, lures, and standardized oviposition traps. Treatments included short-term cold storage of pupae (up to 2 days at 15 °C) and delayed mating through dark storage of adults (up to 7 days). Eggs were collected at fixed two-day intervals until production ceased, and total egg yield (g) per cage served as the primary response variable. Results show that dark storage is a viable way to reduce the number of egg harvests without significantly affecting total egg production, provided the delay does not exceed four days after the first flies emerge. Flies kept under a normal day–night cycle required five harvests to produce an average of 23.2 g of eggs, whereas flies stored in darkness for up to four days required only three harvests and still reached 22.3 g. The results for cold-stored pupae are preliminary, as the third trial is still ongoing at the time of writing. However, the first two trials already showed increased egg outputs of +28% and +12.7% compared with non-stored pupae. These findings demonstrate that interventions at different life stages can improve both operational efficiency, by enabling better alignment with production schedules and reducing the number of egg harvests and, in some cases, even enhance overall egg output. Short-term pupal cooling and controlled dark storage thus offer practical tools for optimizing BSF reproductive performance.

Ammonia emissions during BSFL rearing: initial continuous measurements and the impact of feed protein/carbohydrate ratio

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All animals produce emissions. Hence, there are emissions generated during the rearing of black soldier fly larvae (BSFL). Most notably carbon dioxide and ammonia result both from the microbial activity in the feed for the larvae as well as the larvae themselves. Especially in western Europe the emissions released due to animal husbandry are a hotly debated item. However, for mini livestock, such as the BSF, the available information is very scarce. Within the HiGa project we attempted to start filling this knowledge gap. In the first phase three measurement devices were built and tested to ensure accurate and continuous (every 30s) readings for temperature, humidity, CO2 and ammonia in both the incoming and outgoing air. Currently three devices are operational, and each can measure the emissions of one 60*40cm crate with a variable ventilation speed between 0.1 and 9 m^3/h. In a second stage, and as proof of concept, we investigated how altering the dietary protein-to-carbohydrate (P/C) ratio influences CO_2 and NH□ emissions during BSFL rearing. The control diets consisted of wheat bran and maize flour. In the experimental diets, the maize flour was replaced with soybean meal to change the P/C ratio. All experiments were performed at room temperature of 27 °C and relative humidity of 55 % with 5-day old BSFL (15 000 per 60*40 crate). The initial data clearly indicates that there is a clear positive correlation between the ammonia production and increase in protein in the feed mixture. Even with very small additions. This strengthens the prevailing theory that more proteins in the feed result in more nitrogen losses through volatilization and highlights the importance of optimizing feed P/C ratios to minimize environmental emissions and improve nitrogen utilization efficiency in BSFL production systems. However, further research is needed if this correlation also holds when using other sources of protein such as egg or milk protein.

Computer vision for weight estimation and activity levels in Tenebrio molitor: Improving production and welfare

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Edible insects have emerged as an alternative protein source. They require less land and water, emit fewer greenhouse gases, and are rich in protein and healthy fats. Despite its growing acceptance in Europe, industrial challenges, including regulations, safety, and public perception, remain. A major bottleneck is standardising monitoring systems, especially the accurate measurement and tracking of production and welfare traits. Monitoring weights and assessing movement activity is important because they are key indicators of welfare in animal production. However, current assessments of these traits are mostly manual, requiring time-consuming tasks such as weighing, counting, and manual human observations due to their small size, large numbers, and fragility. Although vital for safety and productivity, manual data collection is labour-intensive, error-prone, and hampers scalability. This study aims to develop a non-invasive computer-vision-based approach using the object detection model YOLO and the foundation model Segment Anything (SAM) to detect, segment, and subsequently estimate the weight and activity levels of yellow mealworms. Larvae were reared under standard conditions (27°C, 65% RH, 0:24 light: dark). For model training and analysis, 226 single-larval and 98 group-larval videos were recorded with 5 larvae per group using a dome IP camera. For both single- and group-larvae videos, segmentation masks were generated for each larva, and the median of the estimated segmentation area was computed. Additionally, for the group videos, larval activity was determined by calculating the displacement of the bounding box centres between frames. After feature extraction, Pearson correlation was used to estimate the linear association between the median of the estimated segmentation areas and the actual larval weight in the single-larva dataset. Subsequently, a linear regression model was fitted using the ground-truth weights and segmentation data to quantify this relationship and generate weight predictions for the larvae in the group videos. Preliminary results show a strong, positive linear relationship between the estimated segmentation masks and ground truth larval weights. Median pixel values correlated with the actual weights at $r = 0.9750$. The median-pixel values explained about 95.1% of the variance with a residual error of 13.91 mg. Our study offers a promising framework for automated assessment of larval weight and activity, which could aid in monitoring key welfare and production traits, such as disease incidence and growth rate. Thus, also a scalable alternative to manual data collection that could minimise human error and facilitate breeding strategies. Further validation is, however, needed for continuous monitoring in enclosed production systems, especially regarding how changes in lighting affect stress and welfare detection. Future research on group monitoring under various lighting conditions could improve non-invasive assessment of yellow mealworms.

Waste Valorization Through Insects: Larval Growth Evaluation of Hermetia illucens on Olive Pomace Diets

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Two-phase olive pomace (TPOP) is the main byproduct of olive-oil extraction process, accounting for more than 80% of the raw material. Finding effective ways to convert this residue is important, as its uncontrolled disposal leads to soil and water pollution, increased phytotoxicity, and reduced water infiltration. Black soldier fly (BSF) larvae are known for their ability to process a wide range of organic byproducts, making them a promising option for TPOP valorization. This study examined the impact of diets consisting of chicken feed and TPOP on BSF larval growth and performance. Eleven diets were prepared using both dry and wet TPOP. Dry TPOP was included at 10, 15, 25, 50 and 75%, while the wet form was included at 5%, 10, 15, 25, 50 and 75%. Chicken feed served as the control diet. Larval survival and weight were recorded every three days, whereas the experiment was completed when 10% of the larvae reached the prepupal stage. In addition, bioconversion efficiency corrected for residue (BER), feed conversion ratio (FCR), waste reduction rate (WRR) and specific growth rate (SGR) were also calculated. According to the results, the highest final individual larval weight was recorded in the control diet (144.0 mg), followed closely by the 25% wet TPOP diet (139.6 mg). The lowest weight was documented in the 75% dry TPOP diet (84.4 mg). Survival remained consistently high across all treatments, exceeding 94% at the end of the trial. According to feed indicators, the 25% wet TPOP diet performed best, showing the highest BER (13.2%) and the lowest FCR (7.6), followed by the 25% dry TPOP diet (11.4% and 8.8, respectively). Overall, these results show that TPOP can be incorporated into BSF diets at moderate levels, supporting its potential use in circular bioeconomy strategies. This research is supported by the EU-PRIMA program – project OLIWA- Repurposing OLIve WAste in circular economy solutions for feeds, additives, packaging, and biogas – GA 2432, Call 2024 Section 1 Food Value Chain in Nexus.

Farming insects: evaluation of safety and legal framework

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Dipteran insects such as black soldier fly larvae (BSFL; Hermetia illucens) are known for their ability to convert a wide range of organic residual streams, including materials that are currently not allowed under EU feed legislation, into valuable biomass. These streams are often excluded because of elevated contaminant levels or the presence of animal by products, which may pose risks of transmitting diseases to humans or animals. However, these residual streams represent valuable opportunities for circularity and valorization. Over the past years, we have conducted multiple experimental studies to evaluate BSFL performance and safety outcomes on a variety of non-authorized, or otherwise unsuitable substrates. This contribution provides an elaborate overview of our research on chemical and microbiological hazards across several projects investigating residual streams contaminated with: packaging materials, microplastics, mycotoxins, pesticide residues, heavy metals, dioxins, food pathogens, parasites, viruses, and antimicrobial resistant genes. Results from these studies are linked to the current regulatory framework for farmed insects and potential changes are discussed. This knowledge will contribute to necessary legal reforms required to permit insect rearing on organic residual streams.

Telomere Length as a Biomarker of Health in Apis mellifera

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Honey bees (Apis mellifera, L.) are indispensable for global agriculture due to their critical role as pollinators, ensuring crop productivity and ecosystem stability. Despite their importance, honey bee populations are increasingly threatened by multiple stressors, including habitat degradation, pesticide exposure, pathogens, and climate change, resulting in significant colony losses worldwide. Recent advancements in molecular biology have introduced biomarkers as valuable tools in agricultural and ecological research, offering innovative approaches to monitor the health and well-being of pollinators. In honey bees, genomic biomarkers are particularly promising due to their capacity to reflect complex environmental interactions and physiological states. Among these, telomere length has emerged as a potential biomarker of health, as demonstrated in other species, including mammals. Telomeres, the repetitive DNA sequences located at the ends of chromosomes, play a vital role in maintaining genomic stability and have been associated with aging, stress response, and overall health. Although telomere sequences are evolutionarily conserved, species-specific methodologies and primers are necessary for accurate measurement. At present, no standardized protocols exist for assessing telomere length in honey bees. This study aims to establish a reliable qPCR-based protocol to quantify telomere length in honey bees, using GAPDH as an internal reference gene to ensure consistent and accurate results across variable conditions. By applying this protocol, we aim to evaluate telomere dynamics in response to environmental stressors, providing new insights into the health and resilience of these essential pollinators. This research is supported by the project "Studio trans-disciplinare per la valutazione della salute dell'ecosistema e degli impatti sulle api in ottica di planetary health" – izs me 02/24 rc.

House cricket (Acheta domesticus) powder as an alternative protein source in the development of protein energy bars

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Edible insects are an alternative source of protein whose production promotes greater environmental sustainability, requiring less space for domestication and fewer resources for production, and causing less environmental pollution than conventional animal production. Therefore, the development of insect-based products with desirable nutritional and sensory properties is necessary to increase animal and human consumption of this products. This study evaluated the potential use of house cricket (Acheta domesticus) powder as an alternative in the production of protein energy bars for human consumption. Three cereal bars were formulated with cricket powder (CP): 0% (without CP – control), 5%, and 10%, incorporating fig paste, puffed rice, sesame seeds, sliced almonds, almond butter, and honey. After being baked (180 °C for 15 to 20 minutes), the bars were cooled and stored for later analysis of the following parameters: water activity (aw), minerals, protein, total fat, fatty acid profile, and total dietary fiber, as well as microbiological analysis. The aw values were always below 0.6, suggesting microbiological stability. The addition of 10% CP increased the ash content. However, the levels of potassium and magnesium did not change significantly with the addition of CP. On the contrary, sodium levels increased. The control formulation (0% CP) had a protein content of 15.1%, f.w., which increased to 17.6% and 20.9% after the addition of 5% and 10% CP, respectively, these latter being considered a source of protein, as well as of fiber (>3 g of fiber/100 g of product (f.w.)). The bars developed in this study exhibited high MUFA and PUFA values (>80%), with oleic (C18:1n9c) and linoleic (C18:2n6c) fatty acids being the most predominant. The developed products presented satisfactory microbiological quality. This study demonstrated the potential of incorporating cricket powder into foods, such as snacks, as a sustainable and nutritious alternative, rich in protein and fiber.

Improving genomic prediction of juvenile mortality through causal variant inclusion in the Normande dairy cattle breed

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Juvenile mortality is partly driven by recessive genetic defects, making it a relevant target for genomic selection. However, standard single-step GBLUP (ssGBLUP) models do not explicitly account for causal variants or their non-additive effects. Therefore, this study consisted of, first, the development of three ssGBLUP models for juvenile mortality that explicitly included a causal variant. These models either assigned (i) a higher proportion of genetic variance explained a priori by the causal variant or (ii) estimated and incorporated its additive or (iii) both additive and dominance effects as covariates. Second, a comparative analysis was performed to identify the most accurate model between the latter and a standard ssGBLUP model, used as a reference. This methodology was applied to the Normande dairy cattle breed where cases of Alopecia of the body extremities have been reported. This recessive genetic defect is caused by an inframe deletion of the replication factor C subunit 5. Homozygous mutant calves exhibit stunted growth, chronic diarrhea, a noticeably thin hair coat, and alopecia affecting the extremities, resulting in a higher incidence of late juvenile mortality (up to 3 years of age). 497,010 female juvenile mortality records were included in a training set (born in 2009-2015), of which 28,150 were genotyped (27,078 live, 1072 dead). A value of 100 was given to animals that died between days 3-1095, and 0 to those alive after day 1095. The validation set consisted of records of 333,476 daughters (born in 2016-2022) of 346 genotyped bulls (13 heterozygous HT and 333 homozygous wildtype WT for the causal variant) considered as candidate bulls in the training set (e.g. bulls without daughters' records). The estimated breeding values (EBVs) of the candidate bulls were estimated with the four models using HSSGBLUP program. The moderate correlations between the bulls' EBVs and the observed mortality rates of their daughters in the validation set indicated similar predictive abilities across the models (R=0.41-0.44). However, EBVs obtained from models that included the causal variant showed a greater difference between homozygous WT and HT compared with the reference model (ranging from +0.51 to +0.57 genetic standard deviation vs +0.32 for the reference model), resulting in an improved ability to differentiate carriers from homozygous WT bulls. Accordingly, integrating causal variants into routine genomic evaluations may offer a valuable strategy for reducing juvenile mortality. Moreover, expanding genotyping efforts to include more dead animals could further improve model performance. Finally, additional research is required to identify the most effective approach for including causal variants into ssGBLUP models.

Insights into the genetic basis of emerging health and vitality related traits in Holstein Friesian calves

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Functional traits including health and vitality are of utmost interest in modern dairy cattle breeding as they ensure vital animals and offspring, leading to genetic merit, reduced cost for treatment and improved animal welfare. Diseases occurring during the early live stages increase veterinary costs, require additional management efforts and may negatively affect performance later in life. In recent years, calf housing and welfare have received increased attention and are ongoing part of scientific and public discussions, focusing on the potential for transformations within the sector. A national prevalence study (PraeRi) conducted by the University of Veterinary Medicine Hannover assessed calf health from 2016 to 2019 on 765 German dairy farms and found an average mortality rate of 5.4 % (retrospective for one year). The most common health issues in calves were navel infection, diarrhea, pneumonia as well as multimorbidity. Although the importance of successful calf rearing has attracted attention in dairy farming practice, traits directly addressing calf health and vitality are not yet adequately considered in breeding programmes and the genetic background of these traits remains largely unknown. The aim of this study is to analyse the genetic architecture of different calf traits and to evaluate their potential for the integration of these traits into future breeding goals through the development of a new trait complex defined as “calf vitality”. The dataset contains more than 3,800 female Holstein Friesian calves which were raised on a large dairy farm in eastern Germany. Phenotypic information of all animals is routinely recorded on the farm and contain birth weight, maternal calving ease, serum total protein as well as repeated records for weight development (6 measurements, between day 30 and 310) from birth to first insemination. Additionally, disease events (pneumonia, navel infection and diarrhea) affecting calf health as well as animal losses and its cause are documented. All animals were genotyped using a 50K bovine SNP Chip. Genetic analyses combining the genetic and phenotypic information were conducted, including the estimation of genetic parameters, followed by genome wide association studies. In initial genetic analyses, SNP-based heritability estimates for the traits birth weight (BW), average daily weight gain from birth to day 120 (ADG120) as well as serum total protein (STP) were $h^2 = 0.52$ (SE 0.038), 0.37 (SE 0.058) and 0.29 (SE 0.037), respectively. The results of this study provide valuable insights into the genetic basis of calf health and vitality traits. A profound understanding of the genetic background enables their consideration in future breeding goals and breeding selection decisions. Combining relevant calf traits into a trait complex referred to as “calf vitality” represents a promising possibility to address the increasing importance of genetic improvement focusing on enhancing calf health and robustness. Ultimately, this may contribute to improved animal health and welfare while at the same time improving sustainability and efficiency in dairy farming systems. This work is funded by the German Federal Ministry of Food and Agriculture (BMEL) based on a decision of the Parliament of the Federal Republic of Germany, granted by the Federal Office for Agriculture and Food (BLE; grant number 281C809A21).

Optimizing lesion phenotypes to enable breeding against psoroptic mange susceptibility in Belgian Blue cattle

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The Belgian Blue (BB) breed forms a cornerstone of Belgium's cattle production because of its valuable beef qualities. However, the breed exhibits high susceptibility to psoroptic mange, a skin disease caused by Psoroptes ovis mites, leading to severe dermatitis and major welfare concerns. Notably, substantial individual variation in lesion development is observed under similar environmental conditions, suggesting a genetic component underlying disease susceptibility. A recent large-scale study evaluated ±2,000 animals across more than 100 management groups, using standardized phenotypes (lesion extent and severity scoring). They reported low but non-zero heritabilities for these traits (h^2 = 0.07-0.09) and identified 3 candidate genes possibly linked to disease susceptibility. Building on this work, we have continued sampling for an independent dataset of approximately 650 animals to validate and refine our previous findings. However, practical experience has shown that the detailed lesion mapping and severity scoring are labour-intensive, time-consuming, and difficult to standardize across farms, which limits their scalability for routine implementation in breeding programs. Therefore, we evaluated whether lesion phenotyping can be simplified without compromising the ability to perform genetic analyses and to develop an effective breeding programme. Specifically, this study aimed to determine whether a lesion phenotype based on assessing lesion extent within defined various anatomical regions (head, neck, topline, flank, belly, hindquarters, tail and legs) can distinguish susceptible from resistant animals and capture most of the biologically relevant variation in disease expression. Lesion distribution per severity score was compared between cases (n = 139) and controls (n = 139). Within each management group, cases were defined as the 10% most severely affected animals and controls as the 10% least affected, based on severe lesion extent. Across all scores, cases showed a broader and more severe lesion distribution. The topline, hind quarter, flank, and tail were most frequently affected, consistent with clinical descriptions of psoroptic mange. Severe lesions (scores 3–4) were particularly concentrated on the topline and hind quarter. The flank showed strong discriminatory power, with severe lesions present in up to 50% of cases versus 12.5% of controls. Each dual-trait animal model included total lesion extent as trait 1, while trait 2 was alternated across the different regional lesion measures. Genetic correlations between total lesion extent and the specific regions ranged from −0.12 to 0.87, indicating that some regions (topline, hind quarter and flank) capture a substantial proportion of the genetic variation underlying overall lesion extent. Predictive tree-based modelling further identified the topline, hind quarter, and flank as the most informative regions for classifying case/control status. These findings indicate that region-specific lesion assessment, particularly focusing on topline, hind quarter, and flank, may capture a substantial proportion of meaningful phenotypic variation. A reduced, region-based scoring system could therefore improve feasibility and standardization while maintaining biological relevance for genetic evaluation.

Variance components for number of oocytes and number of embryos in Austrian Fleckvieh cattle

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The total number of oocytes (NoO) and embryos (NoE) was recorded in Austrian Fleckvieh cattle from multiple ovulation and embryo transfer (MOET) sessions conducted between 1991 and 2025. In total, approximately 2,200 records for NoO and 3,100 records for NoE were available from sessions performed on 579 farms. NoO ranged from 0 to 65 oozytes with a mean of 12.2, while NoE ranged from 0 to 50 with an average of 7.6 embryos. Variance components were estimated using the software WOMBAT. The statistical model included age within lactation number and season within year of MOET session as fixed effects, and farm, permanent environmental, and additive genetic effects as random effects. Heritability estimates were 0.35 for NoO and 0.21 for NoE. A high genetic correlation of 0.96 was observed between NoO and NoE, indicating a largely shared genetic background. A genome-wide association study (GWAS) was conducted using GCTA with the MLMA-LOCO approach, in which a genomic relationship matrix constructed from SNPs on all chromosomes except the one under analysis was used to control for population stratification. Significant associations for NoE were identified on chromosome 22. Correlations between estimated breeding values (EBV) for NoO and NoE and EBV for the fertility index, were close to zero (+0.06), suggesting that superovulatory response traits are genetically independent from traditional fertility traits. These results indicate that NoO and NoE show substantial heritability and that both traits could be considered in breeding programs aimed at improving embryo production without adversely affecting fertility.

Revealing inheritance patterns and genomic regions associated with embryonic loss in Holstein Friesian embryos

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Hereditary defects contribute to impaired embryonic development in dairy cattle and represent an important concern for animal welfare and economic losses. While previous studies have inferred embryonic lethality indirectly from adult populations, direct analyses at the embryo level remain limited. In our initial study, single-nucleotide polymorphism (SNP) genotyping data (~50K) from biopsied Holstein Friesian embryos collected between 2018-2023 and derived from elite matings were examined. A total of 865 literature-based candidate markers previously reported to be associated with fertility and fetal viability were pre-selected for analyses. After quality control and application of predefined genotype filtering criteria, 65 autosomal SNP markers were retained; requiring homozygous Q-genotype (aa) frequencies ≤10% in parents, presence of Q-genotypes (>0%) in embryos, and minor allele frequency (MAF) >5% in parents, thereby minimizing potential biases from allele dropout and ensuring robust detection of genotype frequency distortions. The dataset for analysis included 1674 embryos and 715 parental animals. Statistical analyses focused on genotype frequency distributions and deviations from Hardy-Weinberg equilibrium (HWE) in embryonic and parental generations. Among the 65 markers, 55 showed significant deviations from HWE ($p<0.05$) in embryos, whereas their parents remained largely in HWE. Homozygous Q-genotypes were more frequent in embryos (1.9%) than in parents (0.7%). This over-representation of homozygous Q-genotypes in embryos relative to parental expectations indicates recessive inheritance effects on embryo and/or fetal developmental competence to term. The present study aims to validate previously observed genotype distortions on an expanded dataset including embryos collected until January 2025. More importantly, it seeks to expand the genomic scope beyond the literature-based candidate markers to elucidate further the genetic mechanisms underlying embryonic loss and developmental competence to term. The updated dataset comprised 2633 genotyped embryos and 797 genotyped parents. In a first step, same quality control and genotype filtering steps were applied, resulting in the same marker set (N=65). Comparisons of genotype frequency distributions and deviations from HWE in embryonic and parental generations were re-evaluated for these markers. Preliminary results indicate consistent patterns of genotype distortion, with significant deviations from HWE persisting in embryos, whereas parental generations remained largely in HWE. Homozygous Q-genotypes remained overrepresented in embryos relative to parental expectations. These findings support the hypothesis that recessive genotype distortions can already be detected at embryonic stage. Within the analysed marker set, individual SNPs exhibited particularly pronounced genotype distortions. In some cases, homozygous Q-genotypes were observed exclusively in embryos despite their absence in the parental generation. Notably, several of these markers were located on BTA12. Analyses expanding the evaluation to additional genomic regions, to be presented, aim to further refine our understanding of inheritance patterns associated with embryonic loss and developmental competence to term. These resulting findings will contribute to a more comprehensive characterisation of genetic factors affecting embryonic viability and may inform improved genomic management strategies in cattle breeding.

Inferring the genetic background of immunity and associated Vitamin D pathway gene polymorphisms in Holstein cattle

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Advancing genomic selection towards enhanced animal health and immune resilience suggested approaches simultaneously considering phenotypic data from multiple diseases rather than treating them separately. Moreover, specific integration of vitamin D (VitD) pathway genes due to their key roles in innate immunity into targeted selection might contribute to improve the overall animal health status. The aim of this study was to construct an immunity index (IMI) integrating four major diseases across three lactations and to associate signals from genome-wide association studies (GWAS) for the IMI with polymorphisms identified in 16 sequenced genes functionally related to vitamin D metabolism and signaling, with potential relevance for immune regulation. Phenotypes included health records for mastitis (MAST), metabolic disorders (MET), claw disorders (CD) and reproductive pathologies (RP) from 17,437 German Holstein cows kept in 29 large-scale contract herds. Genomic estimated breeding values (EBVs) for the specific diseases were computed using a multiple-trait animal repeatability model. De-regressed proofs (DRP) were calculated for animal i with phenotypic disease records via DRPi = EBVi / ri_2, with ri_2 denoting the EBV reliability. We created an IMI by weighing the DRP according to the health selection index definition as currently applied in official German national genetic evaluations, i.e., IMIi = 0.4DRP(MAST)i + 0.25DRP(MET)i + 0.2DRP(CD)i +0.15DRP(RP)i. The IMI served as dependent variable in GWAS on 50k genotypes applying the leave-one-chromosome-out approach. SNPs with a false discovery rate adjusted P-value below 0.01 were considered as significant, and potential candidate genes located within ±25kb segments surrounding these SNPs were subsequently functionally annotated. The computed IMI ranged from -3.9 (low immune potential) to 1.6 (high immune potential). The GWAS identified 18 significant SNPs linked to genes across seven BTA, playing pivotal roles in VitD-dependent signalling, like CAMK4 which is involved in immune-related transcriptional regulation. Additionally, sequencing of all exons, introns, flanking 5′ upstream and 3'- downstream regions was conducted for the 16 VitD pathway genes ABCB1, CUBN, CYP24A1, CYP27A1, CYP27B1, CYP2J30, CYP2R1, CYP3A28, DHCR7, FGF23, GC, LRP2, NADSYN1, PIM1, SNAI2 and VDR. For this purpose, for a subset including 96 cows, shotgun sequencing was performed with target enrichment on an Illumina NextSeq 2000 platform. The identified variants were mapped to ARS-UCD1.2 and annotated with the Ensembl Variant Effect Predictor tools. The analysis revealed 7,556 SNPs and 766 InDels across the 16 VitD pathway genes. A total of 4,653 detected polymorphisms (60.6%) were previously referenced in the Ensembl variant table, while 3,022 variants were novel. 1.5% of the variants are missense SNPs, e.g.rs447850593 in CYP3A28, leading to p.Arg105Trp which is already linked to milk production and drug residues, and rs109876475 in CYP2R1 leading to p.Val11Ala which is already associated with increased somatic cell count. In conclusion, the developed IMI enabled a comprehensive evaluation of dairy cow immunity. The significant genomic regions for IMI were associated with immune resilience, highlighting the role of annotated VitD pathway genes with functions in VitD-dependent signalling. Targeted sequencing of 16 VitD genes uncovered several functional variants, further supporting the predominant effects of these genes on the overall immune status.

Phenotypic Responses of Milk β-Hydroxybutyrate and Somatic Cell Score to Heat Stress in Dairy Cattle

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Rising temperatures driven by greenhouse gas emissions continue to impact dairy cattle health and productivity. Under heat stress, cows shift priorities toward thermoregulation over essential psychological functions, increasing their susceptibility to metabolic and inflammatory disorders such as ketosis and mastitis. These conditions can be monitored using milk beta-hydroxybutyrate (BHB) and somatic cell score (SCS), respectively. However, Temperature-Humidity Index (THI) thresholds associated with their heat stress responses remain insufficiently characterized. Thus, evaluating BHB and SCS together may provide a more integrated understanding of cattle health under heat stress. The objectives of the study were to: (1) identify THI thresholds at which BHB and SCS begin to respond to heat stress, and (2) quantify their phenotypic changes across several exposure windows. After editing, the dataset provided by Lactanet (collected between 2015 and 2025) included 1,851,881 BHB records and 5,450,830 SCS records from 1,501,009 and 1,753,254 first-parity Canadian Holstein cows, respectively. BHB values were loge transformed (loge (x + 1)) and SCS was calculated based on somatic cell count (SCC cells/mL), as follows: SCS = log2(SCC/100,000) + 3. Outdoor temperature and relative humidity were obtained from the NASA POWER database (https://power.larc.nasa.gov/; accessed 2026-02-05) using herd geographic coordinates. Hourly THI values were calculated and averaged to obtain daily THI values. For each trait, daily THI was aligned with milk test-day records and averaged across seven exposure windows: the day of testing (d0) and cumulative averages from d0 through the preceding six days (d0-d1, d0-d2, …, d0-d6). Single-trait repeatability animal models were fit with fixed effects for herd, year-season of test day, and age-season of calving for both traits. For BHB, days in milk class was also included. Random effects for both models included herd-year of calving, animal, and permanent environment. Residuals of this model were subsequently paired with corresponding THI averages, and a segmented linear polynomial regression was used to estimate THI breakpoints. Coefficient of determination (R_2) was used to identify the most predictive exposure window for each trait by indicating the proportion of variation explained by each model. Mean transformed BHB value was 0.09 ± 0.05 and mean SCS was 2.02 ± 1.60. For both traits, 73% of records originated from Ontario and Quebec. Estimated THI breakpoints differed by exposure window, ranging from 63 to 69 for BHB and 40 to 75 for SCS. The most predictive exposure window for BHB and SCS were d0-d2 and d0-d1 with THI thresholds of 69 (R_2 = 0.93) and 74 (R_2 = 0.84), respectively. These results suggest that short-term heat exposure preceding sampling is associated with changes in BHB and SCS. Across provinces, average summer (June to August) THI was 64. Only 12% of summer days across all provinces exceeded the identified BHB threshold, and 3% exceeded the SCS threshold, suggesting that broader environmental and management factors may play a stronger role on the phenotype than heat stress alone. Establishing robust THI thresholds for BHB and SCS in Canadian Holsteins can help support earlier and more targeted heat-stress mitigation on farm, especially during transition period when cows are most vulnerable.

Characterization of WC1+ porcine γδ T cell subpopulations using transcriptomic data

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Emerging infectious diseases and environmental pressures threaten livestock systems, emphasizing the need to improve animal robustness and to understand the mechanisms underlying disease resistance. γδ T cells represent a substantial proportion of peripheral blood lymphocytes in livestock species and play an important role bridging innate and adaptive immunity. In ruminants and swine, most blood γδ T cells express receptors from the WC1 family. WC1+ γδ T cells can be divided into two main subpopulations (WC1.1 and WC1.2) based on their WC1 gene expression, which correlates with their ability to respond to specific pathogens. However, γδ T cells and their functional segregation in pigs remain poorly understood. Previous studies from our group identified a genomic region in SSCX associated with γδ T cell abundance in blood, and the most significantly associated genetic variant was also associated with higher survival after PRRSV infection. This study aims to investigate the transcriptional profiles and co-expression networks involved in the genetic regulation of γδ T cell abundance in pig peripheral blood. Blood γδ T cell abundance was quantified in 255 healthy piglets by flow cytometry. Genomic DNA was extracted for genotyping using a commercial GGPSNP70 chip and imputed to whole-genome level, resulting in over 8 million variants. Total RNA from whole blood was sequenced and a differential expression analysis between two groups of animals (15 pigs by group) with extreme γδ T cell abundance was conducted. WC1 genes were identified based on bovine orthologues using Ensembl BioMart. A total of 396 genes were differentially expressed, including 253 upregulated and 143 downregulated in animals with high γδ T cell abundance. Functional enrichment of differentially expressed genes revealed strong overrepresentation of immune-related processes and cytokine signaling pathways. Correlation and network analyses of differentially expressed genes across all animals revealed two tightly co-expressed transcriptional modules and identified several genes whose expression was associated with γδ T cell abundance. Genes upregulated in the group with high γδ T cell abundance included, among others, SOX13, WC1 orthologues, GATA3 and MAF, suggesting a WC1.2 Th17-oriented transcriptional program. In contrast, the module downregulated in high-abundance animals comprised key genes including IFNG, TBX21 and CCL5, consistent with a WC1.1 Th1-like cytotoxic program. Furthermore, an expression genome-wide association study detected eQTLs for 74 differentially expressed genes, with strong signals for WC1 orthologues on SSC5 (61–65 Mb), suggesting that local genetic variation may regulate expression within the Th17-like module. These results reveal distinct transcriptional networks underlying blood γδ T cell abundance and highlight WC1-linked gene modules as regulators of Th1 and Th17-like responses, providing potential targets to enhance immunocompetence and disease resistance in swine populations.

Genetic relationship between cardiorespiratory traits assessed via CT imaging with production traits in pigs

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Heart and lung (H&L) traits in pigs are recognized as indicators of overall physiological capacity. Advances in CT imaging technology now enable accurate, large-scale phenotyping of internal organs, offering new opportunities to better understand organ development and the biological relationship between H&L traits with production traits. As breeding goals evolve toward more sustainable, resilient animals, monitoring internal organ development becomes increasingly relevant to ensure that selection decisions maintain optimal physiological capacity in modern production systems. Therefore, the aim of this study was to estimate genetic parameters for H&L traits measured via CT imaging and to evaluate their genetic relationships with production traits in four purebred pig genetic lines. For this purpose, records for H&L volume and weight (n = 52,926) from animals born between 2010 and 2025 were analyzed. Production traits included: wean-to-rear (n = 49,171), and rear-to-finish mortality (n = 92,991), feed intake variation (n = 47,127), stress indicators such as stress score (n = 54,459) and blotchy score (n = 48,075), test daily gain (n = 84,856), percentage of belly (n = 40,267), and number of teats (n = 57,534). The (co) variance components and genetic parameters were estimated using single- and multi-trait animal models using ASREML-R. All H&L traits showed moderate-to-high heritability (h^2 = 0.41 – 0.54). Heart volume and heart weight were perfectly genetically correlated (rg = 1.00), while lung volume and lung weight were also highly correlated (rg = 0.86), suggesting that weight and volume represent the same biological trait for each organ. Genetic correlations between heart and lung traits were close to zero, indicating genetic independence. Heart traits showed small, positive correlations with growth (rg ~ 0.18-0.19) and slightly positive associations with stress- and mortality-related traits (rg ~ 0.9 – 0.14). In contrast, lung traits showed modest negative correlations with robustness-related traits (rg down to -0.36), suggesting improved stress resilience in animals with larger lungs, although they were slightly negatively correlated with growth and belly percentage. Overall, H&L traits are moderately-to-highly heritable, indicating sufficient genetic variation for selection and providing biologically important insights into physiological capacity. The genetic relationships observed with growth, stress, and mortality-related traits highlight the relevance of maintaining an appropriate balance between production efficiency and functional robustness. Although organ size is not considered a primary selection criteria, routine phenotyping using CT technology enables effective monitoring of genetic trends and, if needed, data to facilitate within-line selection for optimal organ development. This approach allows us to continuously monitor physiological capacity while continuing to make genetic improvement in production and robustness traits.

Co-occurrence and genetics of feed intake perturbations and drug treatments in pigs

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Breeding pigs that are resilient to environmental stressors is gaining importance. Recent studies have quantified resilience using deviations from predicted feed intake patterns obtained via automated feeding stations, demonstrating that these feeding perturbation patterns are heritable and genetically correlated with health-related traits, such as treatment incidence. However, the temporal relationship between feed intake perturbations and drug treatments, as well as the underlying genetic architecture of these traits, remains poorly understood. This study used feeding station data recorded at the performance testing station of SUISAG from 2019 to 2025. Daily records of feed intake were available for 7,802 Swiss Large White dam line (SLW) and 2,657 Swiss Landrace (SLR) finishing pigs, as well as medical treatment records and pedigree data. A subset of the phenotyped cohort consisting of 2,717 SLW and 2,052 SLR pigs had microarray-derived genotypes, which were imputed to the sequence-level. Periods with individual perturbations in feed intake were estimated via the R-package UpDown, while correcting for age and breed. Resilience was estimated as the ln-transformed variance of differences in observed versus predicted trait values via linear regression for feed intake (lnvarFI). The total number of medical treatments was summed per pig, discarding group treatments and prophylactic treatments. Co-occurrence of feed intake perturbations and drug treatments was analysed using individual-level binomial mixed-effects models, including a discrete-time hazard framework with daily lead–lag exposure terms. Genetic parameters were estimated per breed using gibbsf90+ software accounting for pen (random), sex and farm of origin (fixed) and the arrival weight and number of daily records (covariate) using single-step GBLUP. Genome-wide association studies (GWAS) between imputed sequence variant genotypes and resilience indicators were performed using GCTA-software. Co-occurrence analysis showed that pigs experiencing a feed intake perturbation had 3.1-fold higher odds of receiving a drug treatment compared with non-perturbed pigs ($p < 0.0001$). Discrete-time hazard modelling revealed a pronounced temporal association, with treatment initiation strongly increased only in the days following feeding perturbation onset, peaking one day after perturbation start (odds ratio = 9.2). Breed effects were not significant, while younger pigs had higher odds of treatment initiation. Heritability estimates for lnvarFI were moderate (SLR: 19.7%; SLW: 19.1%), while heritability for medical treatments was lower (SLR: 5.2%; SLW: 7.7%). The genetic correlation between lnvarFI and medical treatments was only positive with 95% highest posterior density (HPD) estimates larger than zero in SLW (rg=0.42; HPD=0.15 to 0.62), but not in SLR (rg= -0.03; HPD=-0.58 to 0.46). GWAS for the number of medical treatments did only identify several loci reaching the suggestive threshold (1×10^{-5}). For SLW, the most significant association was found on SSC14 (14:124,703,050, $p=3.0\times10^{-7}$). For SLR, the most promising association was detected on SSC7 (7:21,333,895, $p=1.6\times10^{-6}$) near the PRSS16 gene, which is involved in thymic T-cell development. These findings show that feed intake perturbations strongly predict subsequent medical treatments in SLW and SLR pigs, highlighting their value as resilience indicators. Low to moderate heritability and identified genomic regions suggest a genetic basis for these traits that could be leveraged in breeding for robustness, although further validation is needed due to uncertain genetic correlations.

Genomic background of bone-related traits considering a genotype-by-environmental interaction in Landrace boars

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The reduction of nitrogen (N) and phosphorus (P) in animal feed aims to minimise environmental pollution, such as excess nutrients in water bodies and the impairment of soil quality, without impairing the growth and health of the animals. The aim of this work was to investigate the effects of N/P-restricted diets on the morphological bone structure, chemical bone composition, bone density, as well as fundament conformation in pigs. In total 1’082 purebred landrace (LR) boars were split into two feeding groups. The diet of the control group (CON) contained, depending on the live weight, crude protein and P in a range of 187 to 171g/ kg DM (CP) and 6 to 5.5 g/kg DM (P), respectively. The N/P-reduced diet (LPP) contained ~10% less CP and ~7% less P following the recommendations by DLG. Before slaughtering leg and body conformation of 835 boars was evaluated subjectively by a routinely applied linear scoring system. After slaughtering, the right front foot from all animals was collected, and the metacarpal bones 3 (Mc3) and 4 (Mc4) were extracted. Length, weight, and diameter of Mc3 and Mc4 were measured, and the bone density of Mc3 was determined by computer tomography (CT). Afterwards, the content of ash, calcium, phosphorus, magnesium, and sodium of Mc3 was analysed. There was no significant impact of the diet group on bone density. The chemical analyses showed significant differences for the magnesium content only. Four conformation traits were significantly affected by the diet group. Skin or muscle tissue samples were routinely collected post mortem. Genomic DNA was isolated from the collected samples. Single Nucleotide Polymorphisms (SNPs) of all boars were genotyped on the Porcine 70k ggp porcine Illumina© HD array. Quality control was performed using PLINK, accounting for low call rate >0.95 and low minor allele frequency (MAF) >0.01, leading to 55’378 SNP for further analyses. The Genome-Wide Association Study was performed using the rrBLUP. First, these data were analysed by considering the feeding group as a fixed effect in the model. This was followed by an analysis within each feeding group, where the data were divided by feeding group. To correct for multiple testing, chromosome-wide Bonferroni significance levels ($p<0.05$) were calculated based on the number of tag SNPs representing the different chromosomes. A total of 102 SNPs located on 7 autosomes were significantly chromosome-wide associated with at least one bone-related trait. In particular, two regions located on Sus scrofa chromosome (SSC) 14 and one region on SSC 16 were identified as being associated with six bone morphological traits, three chemical composition traits, and two CT-related bone traits. This suggests potential pleiotropic effects. The analyses within the feeding groups revealed 24 and 21 significantly associated SNPs for the CON and the LPP, respectively. Interestingly, the region on SSC16 was only detected in the CON, whereas the region on SSC14 was only significantly associated with bone-related traits in the LLP. This could indicate genotype × environment interactions, whereby a different set of genes is responsible for the expression of bone-related traits depending on the feeding strategy. The next step is to investigate the identified regions for any candidate genes and functionally relevant variants.

A deterministic epidemiological model of flavobacteriosis in rainbow trout farming: implications for farm management and genetic improvement

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The objective of this study was to develop a modelling framework describing the transmission dynamics of flavobacteriosis in aquaculture systems and to identify practical levers for disease control through farm management and genetic improvement. Flavobacteriosis, caused by Flavobacterium psychrophilum, is a major health challenge in rainbow trout (Oncorhynchus mykiss) aquaculture and can lead to severe mortality during outbreaks, resulting in significant economic losses. Effective control remains difficult because the epidemiological mechanisms governing pathogen transmission within aquaculture systems are still insufficiently understood. Current knowledge on flavobacteriosis largely relies on experimental infection studies and challenge tests that investigate host resistance and pathogen virulence (Nematollahi et al., 2003). While these studies provide valuable information on infection processes, quantitative epidemiological approaches remain limited. In particular, modelling frameworks capable of linking disease transmission dynamics with aquaculture management practices and host genetic variability are still scarce (Brenden et al., 2023). We developed a deterministic compartmental epidemiological model describing disease transmission in farmed rainbow trout populations. Fish are represented by epidemiological states (susceptible, exposed, infected, recovered and dead), coupled with the dynamics of pathogen concentration in the aquatic environment. The model incorporates key processes relevant to aquaculture systems, including pathogen excretion by infected and dead fish, pathogen removal through water exchange, and removal of dead fish from tanks. Genetic heterogeneity within the fish population is explicitly represented through variability in epidemiological traits related to infection, recovery and pathogen excretion. Model parameters were compiled and calibrated using experimental results and epidemiological information reported in the literature in order to generate biologically realistic simulations of outbreak dynamics. Model analyses identify conditions under which epidemics can spread within a farm and highlight the influence of management-related parameters such as water renewal rate and carcass removal frequency, as well as epidemiological traits including pathogen excretion and host recovery rates. By linking disease dynamics with both farm management practices and host genetic variability, this modelling framework provides useful guidance for farmers, health managers and breeding programs aiming to improve disease resilience in rainbow trout aquaculture.

Mating strategies to increase the polled phenotype in organic dairy cattle production: potential and consequences

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Following a revision of its branch guidelines, the Danish organic cattle industry has committed to banning dehorning by 2031. Consequently, farmers who wish to avoid horned animals must rapidly increase the proportion of polled (naturally hornless) cattle. This simulation study evaluated the impact, both in terms of genetic gain and genetic diversity, of different mating strategies aimed to increase the frequency of polled animals in organic herds. Simulations started from real haplotype data, including 47 599 autosomal SNPs, from Danish Holstein animals born in 2024. About 65% of animals were horned, 32% polled single carriers and 3% homozygous polled based on genotypes at the polled locus. A dairy cattle breeding program was simulated in which 10 000 cows were mated to 50 bulls each year resulting in 8 000 viable calves. Selection was performed on a single trait, resembling the total merit index, simulated by designating 10% of SNP as QTL whose additive effects were sampled from a normal distribution. Genetic effects were scaled in the founders so that genetic variance was equal to 1. All 8 000 newborn calves were genotyped. Each year, the best 30 1-year-old males were selected to become AI sires with fixed genotype distribution (best 18 horned, 9 heterozygous polled, 3 homozygous polled bulls). Selection was performed using genomic breeding values (GEBVs) sampled to achieve a reliability of 0.50 for candidates without own phenotypes. Twenty additional bulls aged 2-3 years were selected independently of polled genotype. The best 10 000 females were also selected on GEBVs and the top 400 were flushed annually to produce 4 viable calves. All matings were performed at random in the breeding population. An organic population of 5 000 cows was also simulated in which five mating strategies were tested: 1) Baseline with random mating considering all 50 AI bulls; 2) S1: mating include at least one polled parent; 3) S2: all horned bulls excluded from mating; 4) S3: all horned females mated to polled homozygous bulls and polled females to any polled bulls; and 5) S4: identical to S3 but doubling the number of matings allowed for homozygous polled bulls. Simulations ran for 20 years with 20 replicates per scenario. Mating strategies were compared based on the proportion of polled calves born in organic herds, achieved genetic gain and inbreeding trends, and extended haplotype homozygosity (EHH) around the polled locus. The proportion of polled calves born remained stable in the baseline scenario (0.30-0.45) but increased sharply in strategies S3 and S4. About 90% of calves were polled within 5 years in S4 and 20 years in S3. Compared with the baseline, mating strategies S1-S4 reduced genetic gain by 11.8 to 13.1%, because the use of high merit horned bulls was restricted or excluded. Differences in genetic gain were not statistically significant as gain depended largely on the group of heterozygous bulls. Due to low recombination rate around the polled locus, EHH remained high across all mating strategies and was highest for S4. Genome-wide inbreeding trends did not differ significantly among scenarios. Notably, homozygous polled animals remained slightly more inbred than horned and polled single carrier animals over 20 years in all scenarios. In conclusion, rapid adoption of polled genetics in organic dairy herds requires the availability and intensive use of homozygous polled bulls. While targeted mating strategies accelerate polledness, they slightly reduce genetic gain and maintain a strong selection signature at the polled locus. Adjustments to selection and mating decisions, including increased selection of polled bulls and optimization of matings, may help mitigate impacts on genetic diversity.

Establishment of breeding lines for organic dairy production

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Organic farming plays a vital role in agricultural production. The European Union considers organic farming to be a key element in promoting sustainable agriculture; consequently, the EU has set a target of 25% organic agricultural land by 2030. While the management practices and labeling of organic products have been clearly regulated, the origin of animals in organic farms has, to date, been sourced from conventional breeding programs despite existing EU regulations (Regulation (EU) 2018/848). In the future we expect that animals used for organic production need to be sourced from organic farms. Also, we assume that organic dairy producers as well as other organic stakeholders want to differentiate from conventional production with respect to both management procedures and their genetic material used in organic dairy productions. The Danish project "Breeding dairy cows adapted for organic production" aims to demonstrate methods for practical implementation of organic dairy breeding schemes. Consumer (organic and conventional) surveys have been carried out among German, Swedish and Danish consumers. The consumers were asked how much they were willing to pay for organic dairy products. The results showed that consumers were willing to pay most when welfare-traits were included in the breeding goal. Traits related to climate impact were considered less important, while there was nearly no willingness-to-pay for increased milk yield. Furthermore, the survey revealed that consumers knew little about "modern" reproduction techniques. In addition to consumer preferences, we will also derive economic values based on farmer profit using a bio-economic farm model. Economic values are derived for three organic production systems defined by dairy farmers with focus on: 1) Efficiency Improvement within current organic regulations (ECO), 2) Biodiversity with high self-sufficiency and minimal transport and (ECO+) 3) Biodiversity, minimal transport, home grown feed, "naturalness" and "max" animal welfare (ECO++). Economic values and consumer preferences will be combined to weigh traits in the breeding goal with more weight on welfare, climate and product quality traits and less on production. Finally, these breeding goals will be used for designing optimal breeding schemes using the simulation program ADAM, followed by cost-benefit analysis of running breeding schemes for organic dairy production.

Genomic evaluation of a lactation persistency indicator in French dairy cattle breeds

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Today, lactation persistency in dairy cattle is mainly estimated via random regression models on test-day records, providing accurate genetic values for post-peak slope or late-lactation yield. Yet a simple indicator remains valuable: easy to compute and interpret while being low-cost. Better persistency supports longer lactations, delivering economic savings (lower replacement/rearing/vet costs, stable profitability), ecological benefits (reduced Green House gas emissions per kg milk, fewer surplus animals), and welfare gains (less metabolic stress, better longevity and health). To provide references and evaluate genetic potential for extended lactations, we investigated persistency of milk protein yield (PY) in six French breeds: Abondance, Tarentaise, Brown Swiss, Simmental, Montbéliarde, and Vosgienne. Data originated from official milk recording, spanning up to ten lactations. Persistency was defined as the ratio (in %) of PY from days 101 to 200 to PY from days 6 to 100. Genetic parameters including heritability and repeatability coefficients were estimated using the AI-REML algorithm in WOMBAT software. A univariate repeatability animal model included fixed effects of herd-year, age class, calving month, and drying-off status, plus permanent environment, additive genetic, and residual random effects. Correlations of persistency genomic predictions with national genomic estimated breeding values (GEBVs) for other traits were also computed. Persistency ranged from 0% to 195%, mostly between 50–130%, indicating strong phenotypic variation. Phenotypic mean varied between breeds, from 82.1% for Vosgienne to 93% for Brown Swiss, suggesting different improvement potential across breeds. Persistency performances increased over time in five breeds reflecting long-term phenotypic improvement likely driven by selection on correlated traits. In contrast, the Vosgienne breed remained stable, suggesting distinct underlying dynamics. Permanent environment exceeded genetic variance. Heritability estimates were low but significant (0.05–0.12 across breeds) and the genetic standard deviation ranged from 4.8 to 9.5, implying potential of improvement. Repeatability was moderate to high (0.30–0.50, lowest in Brown Swiss), indicating consistent individual differences across lactations. Genetic trends were positive in all breeds, accelerating from the 1980s in parallel with intensified multi-trait selection on production and functional traits. Despite the absence of direct inclusion of persistency in the current breeding objectives, these favourable trends demonstrate indirect genetic progress via correlated responses to selection on milk yield and functional traits. Genetic correlations with national GEBVs for other traits were mostly favourable: strongly positive with milk production (0.85-0.90), positive with udder health traits (0.20–0.80), neutral to positive with fertility and calving ease (0.00-0.60), variable for non-return rate. However, its correlation with individual genomic relationship with the rest of the population is negative, (−0.30 to −0.45), showing that genetically original individuals have a lower persistency. These findings indicate that direct selection on this lactation persistency indicator offers interesting potential, given significant heritability and favourable correlations with key production and functional traits. Owing to its simplicity and the indirect progress already observed, the indicator is useful to incorporate into breeding goals to promote more resilient cows, extended lactations, and enhanced sustainability in French dairy breeds.

Prediction of GEBVs for health traits in Australian and German Holstein cattle using Bayesian methods

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Genomic estimated breeding values (GEBVs) are commonly calculated using one of the two main approaches: (i) Genomic Best Linear Unbiased Predictor (GBLUP), and (ii) Bayesian methods. Even though GBLUP is still the most commonly used approach in national evaluations, recently Bayesian approaches have gained more attention. This is especially the case for traits controlled by a few major QTLs in addition to a polygenic effect, and traits with lower heritability, such as health traits. In Germany, health traits have been incorporated as a part of a balanced breeding goal in 2021, and their GEBVs are calculated using a single-step breeding value estimation model (ssGBLUP). However, to our knowledge, Bayesian methods haven't been commonly applied to calculate GEBVs on these traits or to evaluate the performance of the two approaches. By the end of 2025 the German national evaluation dataset contained 460,000 genotyped female cows with phenotypes for various health traits. Cow genotype data was collected during the KuhVision project, the purpose of which was to build a female reference population for the calculation of GEBVs. Part of this dataset was imputed to whole genome sequence, and SNP effects were calculated for various complex traits using a mixed linear model approach. To utilize the availability of large-scale WGS dataset in the prediction of EBVs using Bayesian methods, we first estimated the SNP effects of more than 2 million markers from ~100,000 to 180,000 German Holstein cows in 11 health traits using BayesR3–Bayesian MCMC mixed-effects genetic model. Health traits included six feet and leg related diseases; claw ulcers, digital phlegmon, digital dermatitis, interdigital hyperplasia, laminitis and white line disease, three metabolic diseases; milk fever, displaced abomasum, ketosis, and mastitis and somatic cell score. SNP effects calculated here will be correlated with EBV for some health-related metabolic traits in Australian genotype data.

Early Good Is Not Late Good: Why Early Performers Are Not Necessarily Finishing-Phase Winners

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Growth impairments in pigs are economically and clinically relevant but often detected late. Although growth curve models such as the Gompertz function can more accurately capture growth trajectories and health-related deviations, they require multiple measurements per animal and are therefore rarely feasible under routine farm conditions. This study examined whether a consistent performance ranking can be derived from few weighing events across the nursery and finishing phases, or whether phase-specific rank shifts occur that necessitate separate evaluation of both production stages. Individual body weights were collected in a commercial herd. The first weighing (W1) was conducted shortly before weaning, the second (W2) at the end of the nursery period, and the third (W3) was based on the calculated gross live weight of the animal at slaughter (421 pigs; 1263 weight records). The nursery phase (P1) was defined as W1→W2 and the finishing phase (P2) as W2→W3. To quantify phase-specific performance independently of starting level and time, expected end-of-phase weights were derived from batch-referenced linear models accounting for initial weight, time and batch structure (P1: $\hat{W}2$ from W2 ~ W1 + dt12 + batch; P2: $\hat{W}3$ from W3 ~ W2 + mast_days + batch). Performance deviations were then expressed as residuals, defined as observed minus expected weight (rP1 = W2 − $\hat{W}2$; rP2 = W3 − $\hat{W}3$), enabling identification of under- and overperforming animals within a batch. Residual distributions were centered close to zero (medians: rP1 +0.04 kg; rP2 −0.14 kg), supporting their interpretation as relative, within-batch performance deviations. No animal exceeded the plausibility threshold in either phase; maximum deviations corresponded to approximately −197 g/day (nursery) and +176 g/day (finishing). Only a weak association was observed between nursery and finishing deviations ($r = -0.06$; $p = 0.22$), indicating pronounced phase-specific re-ranking and compensatory growth patterns. Consistently, pigs in the lowest rP1 decile required a median of approximately seven additional days to reach slaughter (W2→slaughter). These findings demonstrate that a small number of weighings enables practical, phase-specific monitoring of performance development, but a stable ranking across nursery and finishing cannot be assumed. The approach supports within-batch prioritization (identification of outlier animals) and phase-specific interpretation of performance patterns ("lost early," "lost late," "caught up"). Future work will validate the method in additional batches and evaluate predictive performance in true within-batch train/test setups. In the longer term, deviations can be contextualized by integrating routinely available process indicators (e.g., barn climate, feed intake, health events) to support identification of potential drivers of phase-specific performance loss.
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Identification of candidate genes and key QTL associated with anogenital distance in Holstein cattle

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Anogenital distance (AGD) is the distance from the centre of the anus to the base of the clitoris in dairy cows and has been proposed as an early-in-life indicator of fertility. Shorter AGD has been phenotypically associated with earlier return to cyclicity, shorter calving-to-conception intervals, and higher pregnancy rates. Because prenatal androgen exposure lengthens AGD, the trait may capture variation in fetal androgen signaling. Given its moderate heritability (0.37±0.08), AGD is a promising phenotype to improve selection for female fertility. However, its genomic architecture in dairy cattle remains uncharacterized. The objective of this study was to identify genomic regions, candidate genes and functional pathways associated with AGD in Canadian Holsteins using a single-step genome-wide association study (ssGWAS). Phenotypic records were available for 5,541 heifers and cows from 20 herds. After data editing, 4,988 animals remained for analysis. Genotypes from 6,631 animals in the pedigree and 40,274 single nucleotide polymorphisms (SNP) located on autosomal chromosomes passed quality control. A single-trait animal model was implemented in the BLUPF90 family of programs, integrating both pedigree and genomic information via the hybrid H matrix. The model included fixed effects of technician, quadratic regression on age at measurement, linear regression on days since calving at measurement, and herd-year-season of birth. Significant markers were identified using a chromosome-wise Bonferroni threshold based on the number of independently segregating genomic regions. Additionally, sliding windows of 10 adjacent SNP were used to estimate the proportion of additive genetic variance explained, applying an empirical threshold of ≥0.2%. Gene and QTL annotation were performed within ±50 kb of each significant SNP. A total of 34 significant SNP were identified, with 7 of those located within 6 genomic windows explaining ≥0.2% of the additive genetic variance. A total of 40 unique protein-coding genes were annotated within ±50 kb of each significant SNP that surpassed the chromosome-wise Bonferroni threshold. Among these, STOX2 and STOX1 genes have been implicated in preeclampsia, a pregnancy-associated hypertensive disorder in which elevated androgens may play a role; the CCAR1 gene is a known co-activator of the androgen receptor, which may influence androgen signalling during fetal development; and the NR2C1 gene, one of the genes found to be significant as per consensus of the two analyses, functions as a nuclear receptor that modulates androgen receptor activity and suppresses estrogen-mediated transcription, supporting a potential role in sex steroid signaling. Additionally, 106 nearby QTL previously associated with fertility, puberty, growth, carcass traits, and lactation persistency were also identified. Consensus analysis highlighted 6 genes and 19 QTL supported by both single-marker and window-based approaches. Thus, this study provides novel insights into the genomic architecture of anogenital distance in Canadian Holsteins, identifying significant genomic regions, potential candidate genes, and QTL associated with steroid hormone signalling and reproductive physiology, improving our understanding of the biological mechanisms underlying variation in AGD.

Genomic Diversity and a Haplotype Imputation Reference Panel for Pikeperch (Sander lucioperca)

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Pikeperch (zander; Sander lucioperca Linnaeus, 1758) is an economically important freshwater and brackish-water fish native to the Black, Caspian, and Baltic Sea basins. Its aquaculture has progressed from reliance on wild spawners to reliable controlled reproduction with improved larviculture and recirculating aquaculture systems (RAS) grow-out. However, the breeding of pikeperch lacks genomic resources required to accelerate genetic gain and support broodstock management. To facilitate the development and usage of genomic tools for pikeperch selective breeding, we aimed to 1) characterize genomic diversity in the breeding stock; 2) construct the first species-specific haplotype reference panel that enables imputation from low-density SNP arrays or low-pass sequencing (LPS) to whole-genome sequencing (WGS) resolution by integrating in-house and public datasets. We generated de novo WGS data for 60 samples, including 48 individuals from four breeding cohorts and 22 founders from the base population at Aquapri Innovation (Vejle, Denmark). These were combined with 365 publicly available WGS samples from Germany. Variants calling was performed using GATK best- practices pipelines. Population structure was assessed using principal component analysis (PCA), admixture analysis, and phylogenetic inference. A species-specific reference panel was constructed. We identified approximately 7.4 million bi-allelic variants segregating across 425 samples. PCA revealed clear genetic separation between Danish and German populations. Both the admixture and phylogenetic analysis supported population differentiation between Danish and German samples while indicating shared ancestral origin. Within the Danish samples, the founder individuals (22 individuals) were genetically interspersed with breeding cohorts (48 individuals), indicating genetic contribution of the base population across all cohorts. Based on this variant dataset, we constructed the first haplotype reference panel for pikeperch providing genomic resources for whole genome imputation and downstream breeding applications. This study establishes a foundational genomic diversity framework and delivers the first imputation resource for pikeperch, enabling genome-wide association studies (GWAS), genomic selection, and broodstock management. Ongoing validation using ~2,000 LPS samples will support scalable, cost-efficient selective breeding and long-term genetic improvement in pikeperch aquaculture.

Sequence-level genome-wide analysis of hoof disease traits in Swiss dairy cattle populations reveals a polygenic and breed-specific architecture

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Background: Hoof diseases pose a significant health and welfare challenge to dairy cattle, negatively impacting their productivity and longevity. However, their genomic architecture is not fully understood. Objectives: We aimed to identify possible quantitative trait loci (QTL), candidate genes, and candidate variants associated with infectious and non-infectious hoof diseases in Swiss Holstein (HO) and Brown Swiss (BS) cattle using standardized hoof health phenotypes from routine professional hoof health recording and imputed whole-genome sequence (WGS) single- nucleotide variant (SNV) data. Materials & Methods: Genotypes were quality controlled and imputed to WGS level. After breed assignment and filtering, 87,806 HO and 52,202 BS animals were retained (36.3 and 24.1 million biallelic SNVs, respectively) for further analyses. Phenotypic data comprised standardized hoof health records from professional hoof trimmers. The considered traits included: digital dermatitis (DD), white line disease (WLD), other infectious diseases (INF), and claw horn lesions/diseases (CHL). Yield deviations (YD) were calculated by correcting phenotypes for fixed and random effects. Only YD from the first lactation were then retained and averaged per animal to serve as proxy phenotypes in in genome-wide association studies (GWAS). SNV-based additive heritabilities (h^2) were estimated using GCTA software. Recessive, but also additive GWAS were performed separately using GCTA with breed-specific significance thresholds based on PLINK software pruning and Bonferroni correction. Significant regions were refined using joint analysis and Bayesian fine mapping with PAINTOR software. Candidate variants were prioritized based on statistical significance, PAINTOR credible-set inclusion, linkage disequilibrium with lead SNVs, and biological plausibility of nearby genes. Results: Additive h^2 were generally low across traits and breeds, with the highest estimate for DD in Holstein ($h^2 = 0.086 \pm 0.023$). Additive and recessive GWAS identified multiple breed- and model-specific QTL for the four hoof disease traits. For DD in HO, we identified one single additive QTL on chromosome (chr) 21 but also a recessive QTL on chr 23. In BS, DD was associated with one additive QTL on chr 23 and two recessive QTL on chr 10 and chr 23. For CHL, one additive QTL was detected in HO on chr 16 and one additive QTL in BS on chr 14. Conclusion: This study is the first to analyse the genetic architecture of hoof disease traits in Swiss dairy cattle, revealing a low h^2. Additive and recessive GWAS results using proxy phenotypes suggest a polygenic basis and identify several QTL. One of these QTL was previously found in a different HO cattle population. Our findings require further functional and clinicopathological validation and demonstrated that precise phenotypic data and extensive WGS data are essential for the analysis of health traits with low h^2.

Understanding dairy producer's perspective and experience with reporting gestational and developmental dairy calf conditions

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Genomic selection has accelerated genetic progress in the dairy industry by enabling earlier selection decisions and increasing evaluation accuracy. At the same time, it has contributed to higher rates of inbreeding and reduced genetic diversity. Higher homozygosity increases the likelihood that an individual will inherit two identical-by-descent copies of deleterious or lethal recessive alleles, raising the risk of affected gestational and early calf development, including embryonic loss, stillbirth, congenital deformities, and early-life disorders. As many of these conditions are caused by recessive inherited haplotypes, early identification is essential to limit their frequency in the population. Canada currently lacks a centralized system for reporting and coordinating information on gestational or developmental conditions for downstream analysis. Producers may report to veterinarians, semen distributors, or breed associations, or may not report at all, resulting in a fragmented approach that hinders early detection of emerging genetic issues. To inform the design of an effective national reporting system, an online questionnaire was distributed across Canada through industry sector magazines and producers' meetings. The objective of this study was to characterize producer perspectives on reporting conditions and genetic testing. The questionnaire, available in English and French, included 32 questions organized in three sections: 1) demographics; 2) awareness and comfort with reporting systems; and 3) experience with gestational and calf developmental conditions and reporting behaviour. A total of 130 respondents consented to analysis. Overall, 81.6% of respondents indicated being comfortable with reporting conditions; those who were uncomfortable cited time constraints and uncertainty about where to report. Respondents indicated that a simple electronic format and clear educational resources would improve participation. If the reporting system were improved, 93% of respondents said they would be likely or very likely to use it, highlighting the need to create a reporting system that satisfies producers. Although 87% of respondents had experienced conditions within their herds, 70% of this group did not report these events, and cases were not investigated to prevent recurrence. These findings identify modifiable barriers to reporting and highlight key features required for a practical, producer-centred system. While the responses represent a relatively small, engaged group, they emphasize the need for a centralized database to support genetic monitoring, reduce stigma surrounding conditions, and improve industry-wide learning. Ultimately, the results provide direction for how a system should be structured to enhance coordinated data collection, enable continued genetic discovery, and strengthen long-term management strategies.

Integrating Long-Read Sequencing and SNP Genotyping to Uncover Genetic Determinants of Stillbirth in Holstein Cattle

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Stillbirth in dairy cattle represents not only a significant economic burden but also a key animal welfare concern, with a demonstrable genetic component that remains incompletely resolved. Large-scale genomic studies in multiple cattle populations have mapped quantitative trait loci (QTL), haplotypes, and chromosomal regions associated with stillbirth and related reproductive outcomes, while also highlighting the complex and multifactorial nature of this phenotype. However, previous studies have largely relied on short-read sequencing, which has limited power in detection of large structural variants (SVs), or array-based genotyping, which often misses deleterious variants with low frequencies due to them being poorly tagged by nearby array SNPs. Additionally, without observing the genotypes of stillborn calves directly, candidate causal alleles cannot be pinpointed. To overcome these challenges, we implement a strategy that combines long-read whole-genome sequencing and SNP array data. Our study uses Nanopore long-read sequences from more than 60 Holstein individuals in addition to 50K-SNP-genotypes from hundreds of stillborn calves and their parents and live-born half-sibs. The sample collection is linked to the development and implementation of a stillbirth monitoring system within the project "Monitoring of Genetic and Phenotypic Trends" (MGPT), which collects extensive phenotypic data to accompany these genotypes. Variant discovery begins with extensive analysis of long-read genomes using both reference mapping and a de novo assembly pangenome graph framework, allowing us to capture a broad spectrum of potentially deleterious variants — especially large SVs and sequences absent from the reference genome that are typically missed by short-read approaches. Using annotated regulatory and functional genome resources, we assess the potential phenotypic impact of these variants, with particular attention to rare alleles of high effect. We then define haplotype blocks from the SNP genotypes and impute the long-read-derived variants into the larger stillbirth and adult cohorts. This imputation-enhanced genotype dataset enables the direct identification of rare recessive variants that are homozygous only in stillbirth cases while appearing in heterozygous form in healthy adults — a pattern indicative of deleterious effects. By including actual genotypes from stillborn calves, we observe deleterious haplotypes directly rather than inferring them solely from missing homozygotes in adult populations. Preliminary results already enabled the first description of an inverted repeat with substantial copy number variation underlying a previously identified QTL on BTA18. Through this comprehensive framework, we aim to refine current understanding of Holstein genomic trends, uncover previously unknown genetic contributors to stillbirth and developmental failure, and ultimately design a risk monitoring system based on parental genotypes. Such a system would support breeding decisions that reduce the transmission of defective alleles and improve reproductive performance in dairy cattle populations.

Identification of novel shared loci across purebred boar lines influencing the onset of puberty

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The onset of puberty (OP) is a valuable trait for pig breeding programs, especially for optimizing reproduction management in boars and gilts. Accurately determining the OP can greatly influence farm reproductive management, productivity, and profitability. However, detecting the OP is tedious and typically involves observing sexual behavior and confirming the presence of viable sperm after ejaculate collection. Alternatively, the OP can be estimated indirectly by identifying the inflection point of sigmoidal growth curves, such as the Gompertz curve. In practice, this last approach is advantageous because pig breeding companies routinely collect large phenotypic datasets on growth and development. A Bayesian hierarchical model was previously developed in our group to integrate a whole-genome prediction model using a 25K SNP panel and a pig growth model that fits a Gompertz curve, incorporating a recursive relationship between daily body weight and daily feed intake records. This model was applied to predict key OP traits, including age at OP (AOP) and weight at OP (WOP). These predicted values showed positive correlations with male reproductive performance, i.e., sperm motility, age at first ejaculate, and trainability, supporting their biological relevance. To further elucidate the genetic architecture underlying these novel phenotypes, we conducted a genome-wide association study to identify genomic regions and candidate genes involved in OP variation. Data were available for 25,686 purebred boars from four lines (Duroc, Landrace, Large White, and Synthetic), including 594,095 imputed SNPs obtained through routine imputation procedures. The set of 25K original SNP were used to construct genomic relationship matrices. Association testing was performed for each line separately on autosomal SNPs with a minor allele frequency greater than 0.05. Quantitative trait loci (QTL) were detected using a Bonferroni-adjusted significance threshold (−log10(P-value) > 6.9–7.0), and candidate regions were defined by grouping at least three consecutive significant SNPs located within 1 Mb of each other. The AOP ranged between 110 and 210 days across different lines, with line-specific averages ranging from 146 to 165 days (SD = 8-10 days). The WOP ranged between 75 and 190 kg, with line-specific averages ranging from 91 to 134 kg (SD = 7-11 kg). In total, eight QTL were identified (1 in Duroc, 2 in Landrace, 5 in Large White, and 4 in the Synthetic line). One QTL was shared between Large White, Landrace, and Synthetic lines, and three QTL were shared between the Large White and Synthetic lines, consistent with their shared genetic background. Notably, one shared QTL on chromosome SSC1 (150.0 Mb) exhibited dominance/recessive effects and was located in the CYB5A gene, which encodes a key enzyme involved in steroidogenesis pathways and has been associated with testosterone levels in multiple human GWASes. Further downstream analyses are required to validate these QTL and to identify potential causal variants influencing the onset of puberty in boars.

Single-step genomic predictions considering QTLs with large effects

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Genomic selection has been implemented in many livestock and aquaculture species. Traditional genomic prediction models such as single-step single nucleotide polymorphism (SNP) BLUP (ssSNPBLUP) assume a priori homogenous SNP (co)variances. Hence, all markers are assumed to explain the same proportion of the trait's genetic variance. However, this assumption is violated in the presence of loci with large effects, such as for traits with large QTL effects. In the presence of loci with large effects, SNPs in linkage disequilibrium with such loci are expected to have a larger impact on the phenotypes than others. Different approaches to model these markers in single-step evaluations have been proposed, but usually, limited benefits are observed. Moreover, it is not clear what the impact of different marker weighting in multi-trait evaluations could be when only some traits are associated with a few QTLs with large effects. Therefore, the aim of this study was to implement and validate several single-step genomic prediction models that account for SNPs linked with QTL with large, trait-specific effects. A commercial rainbow trout (Oncorhynchus mykiss) dataset was available, consisting of ~178,000 individuals in the pedigree, ~8,300 individual genotypes at ~4,500 SNPs density, and ~117,000 phenotypes recorded on nine traits across three different environments (nucleus, challenge, and sentinel). Variance components were estimated using a multi-trait pedigree REML approach (heritabilities ranged between 0.02 and 0.69). A univariate single-step GWAS (ssGWAS) was used to identify SNPs significantly associated with each trait ($p \leq 1 \square 10^{-8}$). Four traits showed significant SNPs. Three single-step models were compared: i) ssSNPBLUP: an unweighted single-step SNPBLUP evaluation, assuming homogenous SNP (co)variances (ssSNPBLUP). ii) w_ssSNPBLUP: a weighted ssSNPBLUP weighing SNP (co)variances based on their posterior probability obtained from the univariate ssGWAS. Weights were defined based on a moving average across five SNPs. Only the four traits with significant SNPs were weighted, while the rest were unweighted. iii) LV_ssSNPBLUP: an unweighted single-step SNPBLUP in which SNP(s) associated with QTL(s) are modelled as a different but correlated trait. All models were validated using a forward-in-time cross-validation approach (LR method), comparing two evaluations: a "whole" evaluation using all data, and a "partial" evaluation in which phenotypes of individuals born after 2019 were masked. Validation individuals were genotyped parents born in 2019 (74 males and 122 females). Within each model, genomic estimated breeding values (GEBVs) from the partial and whole evaluations were compared to estimate dispersion and accuracy. Compared to unweighted ssSNPBLUP, the w_ssSNPBLUP approach improved the accuracy (from 0.56 to 0.69 in males, and from 0.48 to 0.60 in females) and reduced dispersion (from 0.89 to 1.00 in males, and from 0.76 to 0.94 in females) of GEBVs for bacterial cold water disease (BCWD), a trait associated with highly significant QTL effects. For other traits, w_ssSNPBLUP showed no advantage or had a slightly negative impact compared to ssSNPBLUP, likely due to overemphasizing weight on only a few SNPs or genetic correlations between traits. Finally, modelling QTLs as separate correlated phenotypes in LV_ssSNPBLUP showed no significant advantage over ssSNPBLUP. Our results show that for traits with large QTL effects, such as for BCWD in this study, the weighted ssSNPBLUP model yields more accurate genomic predictions.

Evaluation of targeted sequencing as a flexible alternative to SNP arrays for routine cattle genotyping

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High-density SNP arrays are the current standard for cattle genotyping in genomic selection and breeding programmes. While robust and widely adopted, array-based platforms rely on fixed marker content, limiting rapid incorporation of newly discovered variants and reducing flexibility as breeding objectives evolve. Targeted sequencing panels offer the potential to overcome these limitations by enabling dynamic marker selection while maintaining compatibility with established genomic evaluation pipelines. However, evidence for their feasibility, operational scalability, and cost-effectiveness under routine commercial genotyping conditions remains limited. The objective of this study is to evaluate whether targeted sequencing can provide a technically reliable, operationally scalable, and economically viable alternative to SNP arrays for routine cattle genotyping in commercial breeding programmes. Commercial cattle DNA samples (initial dataset n = 96, with ongoing expansion) are analysed using both array genotyping and a bovine targeted sequencing panel developed in collaboration with Twist Bioscience. Sequencing is performed on an in-house Element Biosciences Aviti platform to assess implementation within a practical laboratory workflow. Comparative evaluation includes genotype concordance, call rate, coverage uniformity, data completeness, turnaround time and estimated cost per sample. This study provides an applied framework for transitioning from fixed array-based genotyping towards flexible, scalable sequencing-based strategies, supporting adoption in commercial cattle breeding programmes. Demonstrating comparable data quality combined with improved flexibility and competitive costs would enable faster integration of novel traits and accelerate genetic improvement.

Pangenome construction enables detailed investigation of the IGH locus in alpacas

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Camelid single-domain antibodies, derived from natural occurring heavy-chain-only antibodies (HCAbs), represent a unique class of antigen-binding molecules. Due to their exceptional stability and small size, they have become increasingly important in diagnostics, research, and therapeutic development. While conventional IgG molecules consist of two light and two heavy chains, these specialized IgG subclasses are composed solely of heavy chains and are, therefore, referred to as HCAbs. The ability to produce HCAbs is encoded within the immunoglobulin heavy chain (IGH) locus, where HCAb-associated γ constant genes harbor characteristic structural features that prevent light-chain association. However, the genomic organization, allelic diversity, and regulatory architecture of the IGH locus in camelids, particularly in alpacas, remain incompletely annotated and poorly resolved at the haplotype level. In this study, we generate haplotype-resolved long-read assemblies of the IGH locus across alpacas to characterize structural variation across individuals that are currently not represented in the reference genome. For this purpose, we used blood samples from 10 unrelated alpacas, 5 females and 5 males. To create a pangenome, we performed long-read sequencing using PacBio HiFi to examine the individual differences between the animals in the IGH locus in more detail. We then compared the IGH locus of the alpaca with that of dromedary, for which some variants responsible for the development of HCAbs have already been described, to assess whether these variants are also present in alpacas. Although the IGH locus of alpaca and dromedary show high similarity, not all HCAb-associated variants occur in alpacas. Instead, we identified novel variants that may contribute to HCAb development in this species. Long-read sequencing was essential for pangenome construction because antibodies loci are highly repetitive and rapidly exceed the resolving power of short-read technologies. By spanning the full IGH region, long reads enable accurate assembly and variant detection, resulting in more complete and reliable representation than short-read sequencing. This finding provides an important basis for targeted selection and breeding strategies for HCAbs production.

GENIUS: Genotype-Informed Unbiased Selection of Individuals for Efficient Phenotyping

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The rapid decline in genotyping costs over the past two decades has resulted in an unprecedented increase in the number of genotyped animals across all livestock species. In contrast, phenotyping complex traits, such as health, behavior, resilience, and efficiency, remains expensive and labor-intensive. Consequently, the limited availability of high-quality phenotypic records has become a major constraint for exploring the genetic architecture of these traits. However, the abundance of genomic data creates new opportunities to strategically select the most informative individuals for phenotyping based on their genotype information, an approach we term GENIUS (Genotype-Informed Unbiased Selection). Using a simulated dataset comprising 2,326 individuals genotyped for 10,031 SNPs and segregating three additive quantitative trait loci (QTL), we evaluated the utility of two strategies for identifying informative individuals for phenotyping: (i) a principal component analysis (PCA) based approach and (ii) a redundancy-reduction (RR) approach aimed at maximizing genetic diversity. The performance of these strategies was compared with random sampling. Both approaches achieved higher QTL detection rates than random selection across reduced subsets of 1,000, 500, and 250 individuals. Notably, the RR strategy outperformed the PCA based method and successfully detected all three QTL using phenotypic information from only 250 individuals, approximately 10% of the dataset. These results demonstrate that GENIUS enables efficient identification of phenotyping candidates and provides a feasible and highly effective strategy to substantially reduce phenotyping costs while maintaining strong power to detect QTL in complex populations.

Discovery of methylated regions from nanopore sequence data of pigs

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Epigenetic variation is an important regulatory layer influencing complex traits in livestock. DNA methylation plays a central role in gene regulation, development, and environmental responses. In pigs, breeding traits such as growth, fertility, health, and robustness are influenced not only by genetic variation but also by regulatory mechanisms affecting gene expression across tissues and individuals. Local breeds are generally considered less performant but more resilient to environmental stress which might be reflected in the methylation patterns and levels. Understanding genome-wide DNA methylation variability is therefore essential for explaining phenotypic diversity, environmental epigenetic effects, and regulatory regions affecting economically important traits. Recent advances in long-read sequencing technologies, particularly nanopore sequencing, allow direct detection of DNA methylation at single-molecule resolution. This enables genome-wide characterization of methylation patterns in individual animals and identification of regions with variable methylation. Such regions may represent regulatory hotspots associated with gene control, chromatin structure, or environmental responsiveness. Genomic DNA from individually sequenced Swabian-Hall pigs was analyzed using long-read nanopore sequencing data. Reads were aligned to the Sus scrofa reference genome (Sscrofa11.1), and per-site DNA methylation calls were extracted directly from aligned BAM files using modkit. CpG methylation site calls with a minimum coverage of five reads were retained for downstream analyses. To focus on CpG sites, methylation calls were intersected with genome-wide CpG coordinates derived from the reference genome sequence with customized bedtools makewindows scripts. Complementary strand CpG sites were combined into CpG dyads by shifting minus-strand coordinates and summarizing methylated and unmethylated read counts per dyad. Weighted methylation levels per dyad were calculated as the proportion of methylated reads divided by all reads per dyad. CpG island annotations were obtained from the UCSC Genome Browser. Dyads overlapping CpG islands were aggregated to compute island-level methylation estimates for each individual. In addition, genome-wide sliding windows of 1 kb window size with 50 % overlap, were used to summarize methylation levels across the genome. For each window, methylation fractions were calculated based on the summed methylated and total read counts of all CpG dyads within the region. Per-sample results were combined into methylation matrices for downstream comparative analyses. After filtering, about 90 to 130 k 5mC calls per sample were retained, of which 11 to 19 k per sample intersected with CpG-dyad coordinates derived from the reference sequence. Summary of CpG dyads in sliding windows returned about 5’000 windows, of which one window had a standard deviation of methylation fraction across individuals of 23> 0.3. These windows were located in eight consecutive clusters on seven chromosomes. Intersection of dyads with 3’900 known CpG islands discovered 55 thereof covered with methylation in the studied pigs. Two islands (14:51706840–51732873 and 12:58569822–58570047), the first also supported by the window approach, showed pronounced variance in methylation levels among samples, making them candidates for differentially methylated regions. Our results demonstrate the feasibility of nanopore sequencing to identify methylation variability and candidate regulatory regions as a basis to study phenotypic diversity in pigs.

Seasonal drought reshapes the rumen microbiome and metabolic potential of Ethiopian Boran cattle

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Increasing drought frequency associated with climate change threatens livestock production in semi-arid regions. The rumen microbiome is essential for plant fibre degradation and nutrient utilisation, yet its response to extreme drought conditions remains poorly understood. We hypothesised that drought alters rumen microbial composition and metabolic potential in Boran cattle, an indigenous Ethiopian breed adapted to harsh environments. Rumen samples were collected from Boran cattle during the dry season (February) and the rainy season (April). The study initially included 20 animals, but the severe 2022 Horn of Africa drought resulted in the death of 13 animals before the second sampling. Metagenomic sequencing was performed using Illumina short-read and Oxford Nanopore long-read sequencing. Taxonomic reads profiles were generated independently for both datasets, and only taxa detected by both technologies were retained. Illumina reads were used for quantitative analysis. Hybrid assemblies combining Illumina and Nanopore reads were generated using the MUFFIN workflow to reconstruct metagenome-assembled genomes (MAGs) and proceed with functional annotation and pathway reconstruction. Result visualisation was performed using PANKEGG. Taxonomic reads analysis revealed clear seasonal shifts in rumen microbial composition. During drought conditions, the genera Butyrivibrio and Prevotella were the dominant taxa associated with plant degradation. In contrast, Fibrobacter became the most abundant cellulose-degrading genus during the rainy season. Genome-resolved analysis supported these findings: Fibrobacter MAGs were recovered from all rainy-season samples but were absent from drought samples, whereas Prevotella MAGs were detected in all samples across both seasons. Functional analysis of MAGs showed reduced completeness of starch and sucrose metabolism pathways in drought samples compared with rainy-season samples. At the sample level, pathway completeness increased during the rainy season, with Fibrobacter contributing approximately 25% of the detected pathway genes. Overall, metabolic pathway completeness was consistently lower in drought samples. These differences likely reflect seasonal variation in feed availability and composition. During drought, cattle mainly consume dry shrubs and straw containing higher lignin levels, which may limit cellulose degradation by Fibrobacter. In contrast, Prevotella and Butyrivibrio appear more tolerant to lignified plant material and may dominate under drought conditions. Reduced microbial metabolic potential during drought may therefore decrease feed utilisation efficiency when forage resources are limited. Despite the reduced sample size, this study provides evidence that extreme drought conditions substantially reshape rumen microbial communities and their metabolic capabilities in Boran cattle. Understanding microbiome responses to environmental stress may help improve livestock resilience to increasingly frequent climate extremes.

Independent validation of Enterosignatures reveals reproducible community patterns and stress-associated assembly under porcine commercial conditions

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Porcine gut microbiota represents a highly complex and dynamic ecosystem, whose organization and relationship with host phenotypes are important for animal health and welfare, food security, environmental sustainability, and even biomedicine. The concept of Enterosignatures (ES), defined as commonly co-occurring bacterial guilds, has been recently proposed in a Duroc pig population. The present study aims to independently validate the existence of pig ES across different breeds during the fattening period under commercial production conditions. Furthermore, we explore ES assembly in pigs exposed to prolongate social stress and examine their associations with host performance. To achieve this goal, we conducted a longitudinal study with 114 healthy pigs including 54 females (purebred Large White) and 60 males (purebred Large White and crossbred Large White x Landrace (F1)) housed under either control or social stress conditions (high-density stocking) during the fattening period. Body weight (BW) and fecal samples were collected at three time points across the fattening period. Enterosignatures composition was then determined from the fecal microbiota using Non-negative Matrix Factorization (NMF). Despite differences in genetic background and production systems in the pig industry, our findings confirm that the porcine fecal microbiota during the fattening period can be described by combinations of four reproducible ES. Consistent with pioneering reports, ES assembly was driven by Prevotella, Lactobacillus, Clostridium, and Streptococcus genera, which confirm their key ecological role on community structure, reinforcing their contribution in maintaining porcine fecal functional stability and host-microbiome symbiosis. We replicated previously documented impact of prolongated social stress on ES assembly, which was characterized by an increase in ES-Streptococcus and a decrease in the abundance of beneficial ES-Lactobacillus. Differences in body weight (BW) were observed primarily in females, with the control group weighing 7.4 % more than stressed females. Moreover, we replicated the negative association during the fattening period between host-performance and the abundance of ES-Streptococcus and ES-Prevotella. In contrast, no significant differences were observed in males, likely due to the large observed variability on BWs (SD in stressed males was 12.47 kg in comparison to 8.63 kg in females), since male population consisting of purebred Large White and F1 crosses with Landrace. In summary, our study independently validates the existence of porcine ES and their association with host performance during the fattening period, demonstrating strong reproducibility and highlighting the potential of the ES concept for microbiome-tailored strategies to improve pig health, welfare, and performance under commercial production conditions.

Shotgun Metagenomic Characterization of the Rumen Microbiome in Response to the Bovaer Feed Supplement in Lactating Holstein Cows

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Enteric methane from dairy cattle is a major contributor to agricultural greenhouse gas emissions. Dietary supplements present a promising mitigation strategy, but their specific effects on the community structure and functional potential of the rumen microbiome remain poorly understood. The present study aims to further explore the effects by which Bovaer® 10 can reduce enteric methane emissions by characterising the shift in the structure and function of the rumen microbiome of lactating Holstein dairy cows. A 6-week randomized controlled trial was conducted (Nov-Dec 2024), comprising a 2-week covariate period followed by a 4-week experimental period. Twenty cows were divided into 10 blocks based on parity (2 ± 1), milk yield (43.2 ± 10.0 kg/d), and days in milk (123 ± 15). Within each block, animals were randomly assigned to a control (CTR; n=10) or a treatment group (Bovaer; n=10). The basal diet was formulated to meet the nutritional requirements of the herd. Animals under treatment received a basal diet supplemented with Bovaer® 10 (600 mg/kg DMI). Feed intake was recorded using automated feeding stations (Hokofarm®, The Netherlands). Rumination boluses from animals of 5 blocks (CTR = 5; Bovaer = 5) were collected via non-invasive sampling during the 2nd (covariate) and 5th (experimental) week of the trial. DNA was extracted and subjected to shotgun metagenomic sequencing on an Illumina NovaSeqX platform, generating an average of 81 million raw reads per sample. Reads were quality-filtered, host-depleted, and taxonomically profiled. Microbial diversity was analysed via alpha (Shannon, Simpson, observed richness) and beta diversity metrics (Bray-Curtis's distance) with PERMANOVA. Overall, communities maintained high diversity (Shannon: 5.28 ± 0.61) and substantial richness (636 ± 175 microbial taxa). β-diversity analysis revealed that Bovaer treatment explained 25.3% of community variation (PERMANOVA, $p = 0.01$), while timepoint alone has minimal effect ($p > 0.16$). A significant treatment × time interaction was observed ($p = 0.01$), suggesting divergent temporal trajectories between groups. Moreover, a significant treatment × time interaction was observed for total archaea ($p = 0.03$), where the Bovaer group showed a 23% reduction in archaeal relative abundance from the covariate to experimental period (2.69% to 2.08%; Cohen's d = -0.95), whereas the CTR group exhibited an increase (1.29% to 2.11%; Cohen's d = 1.32). Methanobrevibacter, the dominant methanogenic genus and the primary target of Bovaer, had a tendency treatment × time interaction ($p = 0.06$) with a 31.2% reduction in relative abundance (5.19% to 3.57%; Cohen's d = -0.79) in response to the diet supplement. Particularly, Methanobrevibacter sp. 900314635 was observed to have a 30.5% decrease with the Bovaer treatment. Other archaeal species showed a more stable decline response to Bovaer (1.30% to 0.95%) (e.g., Methanomethylophilus sp001481295). In conclusion, shotgun metagenomics analysis demonstrates that Bovaer® 10 not only suppresses key methanogenic archaea but also redirects rumen microbial development toward a less methane-productive state, indicating that its methane-mitigating effect arises from both direct inhibition of methanogenesis and broader ecological restructuring within the rumen. Keywords: Rumen Microbiome, Bovaer, Ecogenomics

Metagenomic insights into the host–microbiome interaction for genetic mitigation of methane emissions in cattle

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The agricultural sector contributes 56% of total methane emissions in the EU, with cattle responsible for about two-thirds of these emissions. Targeting rumen methanogenic microbes represents a promising and direct strategy for methane mitigation in livestock. Emerging evidence shows that methane production and methanogen abundance are partly under host genetic control, making selection for low-emitting animals with a favourable rumen microbiome a sustainable, long-term solution for climate-smart livestock breeding. To advance low-methane breeding, we investigated the genetic parameters of methane emissions and the host control of the rumen microbiome using data from 500 Holstein cows. All cows were genotyped using a 70K SNP array, and methane emissions were measured using sniffers installed in automatic milking systems. Both 16S rRNA amplicon sequencing and whole-metagenome sequencing were applied to identify key microbial species, genes, and pathways, and to evaluate how microbiome information can improve the prediction of breeding values for methane. We observed, while metagenomic sequencing provides deeper insights into microbial functional potential, it tends to underestimate low-abundance taxa. Conversely, amplicon sequencing captures low-abundance methane-related species more effectively, although it is affected by biases from variable gene copy numbers and PCR amplification. Using the newly developed MetaMAG pipeline (https://github.com/msatti123/MetaMAG_Explorer), we identified 111 novel microbial species, several of which participate in hydrogen-utilizing and fermentation pathways, including acetate, fumarate, succinate, and acetyl-CoA pathway. Notably, the succinate pathway emerged as a key mechanism that may redirect energy flow from CH_4 formation toward propionate production, while the acetyl-CoA pathway supports acetate synthesis, both VFA that provide energy to the host animal. Preliminary analyses indicate that approximately half of the microbiome species (n = 3,493) exhibit heritable abundance patterns, with heritability estimates ranging from 0.05 to 0.56. Among these, 52 species belong to archaea. GWAS identified significant loci for 139 microbial species ($p < 3.45 \times 10^{-9}$), with most associated variants located in regulatory regions, suggesting that host control of the rumen microbiome is largely mediated through gene regulatory mechanisms. Statistical models were evaluated to estimate breeding values for methane emissions by integrating genetic and microbiome information. Furthermore, the models were used to quantify the relative contributions of host genetics and the rumen microbiome to the observed variation in methane emissions. Rumen microbiome explained 0.06-0.38% of the phenotypic variation in methane, though its genetic component was limited. Contribution of rumen microbiome to methane heritability was largest with 16S rRNA amplicon data compared to metagenome sequencing, suggesting that 16S rRNA amplicon data can be useful to incorporate into genomic prediction of methane. The prediction accuracy of methane was doubled when 16S rRNA amplicon data was added on top of genetic marker data. Amplicon-based microbiome profiles outperformed metagenomic data due to better detection of low-abundance taxa relevant to methanogenesis.

Runs of homozygosity reveal genomic regions associated with tropical climate adaptation in tropically adapted taurine cattle

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Locally adapted cattle breeds to tropical environments, such as the Brazilian taurine breed Caracu, represent important genetic resources due to their resistance to heat stress and other environmental challenges. In this context, monitoring genetic diversity is essential, as long-term selection within closed herds can increase inbreeding and reduce variability. Runs of homozygosity (ROH) provide a genomic measure of autozygosity, enabling the detection of inbreeding patterns and the identification of regions potentially affected by selection. The aim of this study was to characterize patterns of ROH and to identify homozygous regions shared among individuals in a closed Caracu herd that has been selected for body weight for more than four decades. A total of 935 animals from the Advanced Research and Development Center for Beef Cattle of the Institute of Animal Science, located in Sertãozinho, São Paulo, were genotyped using the Bovine GGP 100K panel. ROHs were identified based on criteria previously established in studies on the Caracu breed, considering minimum length and marker density. The genomic inbreeding (FROH) was estimated from the proportion of the genome covered by ROH. Shared segments among individuals were evaluated to identify homozygosity islands, defined as regions with ROH overlap frequencies greater than 50% in the population. ROHs were detected in all individuals and across all analyzed chromosomes (BTA), reflecting the demographic history and selection processes of the population. A predominance of short ROHs was observed, with 38% of ROHs ranging from 2 to 4 Mb and 35% from 1 to 2 Mb, suggesting the influence of more ancient inbreeding events. BTA20 stood out by presenting the highest average FROH and by harboring the only homozygosity island identified. This island spanned 2.73 Mb, located between positions 38.26 and 40.99 Mb, encompassing 115 SNPs. The identified region contains relevant genes associated with adaptation to tropical environments, including CAPSL, IL7R, SPEF2, PRLR, AGXT2, DNAJC21, BRIX1, RAD1, TTC23L, RAI14, C1QTNF3, SLC45A2, RXFP3, ADAMTS12, TARS1, and NPR3. These genes are involved in processes such as pigmentation, cellular response to heat stress, physiological regulation, and metabolic mechanisms associated with heat tolerance. Collectively, these genes suggest a contribution to protection against solar radiation, maintenance of cellular homeostasis under high temperatures, and increased environmental resilience. These findings reinforce the importance of locally adapted taurine breeds as sources of adaptive genetic variability. Even under a long history of selection for a single exclusively productive trait, such as body weight, the Caracu breed has maintained genomic regions related to its adaptive traits. Furthermore, this study highlights the potential of using genomic information to support conservation strategies and breeding programs aimed at adaptation to high-temperature environments, especially in the context of current and future climate change scenarios.

Updated genome scan for homozygous haplotype depletion identifies a POMT2 nonsense variant causing embryonic loss and stillbirth in Normande cattle

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Genome-wide detection of homozygous genotype depletion among animals genotyped for genomic evaluation is a proven strategy to uncover recessive loci causing either embryonic lethality or early juvenile mortality. Here, we present an updated analysis based on the largest dataset assembled to date for the Normande breed, including 239,675 genotyped animals with available ancestor genotype information. By analyzing sliding windows of 20 markers across more than 44,000 phased and imputed SNPs from the Illumina BovineSNP50 array, we identified two significant signals. The strongest signal, located on chromosome 11 (one homozygote observed vs. 119 expected; Bonferroni-adjusted Poisson P (Padj) = 1.8 $x10^{-50}$), corresponded to a previously reported missense variant in the CAD gene. The second most significant signal (Padj = 6.0 x10-18) on chromosome 10 showed only one observed vs. 54 expected for its lead haplotype. Reproductive performance was significantly reduced in at-risk matings (carrier × carrier). Conception rate after AI was 41.37% versus 46.47% (χ^2 $p < 0.01$), in controls (carrier sire × non-carrier dam). The 56-day non-return rate declined by 5.6 percent point (60.93% vs. 66.57%; χ^2 $p < 0.05$). These reductions were smaller than the 25% decrease expected under complete embryonic lethality, suggesting incomplete penetrance or variable expressivity of the C10P89 haplotype. In contrast, survival was strongly affected: birth survival dropped from 90.42% to 66.81%, and postnatal survival remained lower at 7 days and 30 days ($p < 0.001$, Bonferroni correction). Most losses were stillbirths, indicating the primary effect occurs in late gestation or the perinatal period. A search for candidate variants, leveraging whole-genome sequences of 5 Normande heterozygous bulls, 154 Normande wild-type bulls, and 1,006 individuals from 19 additional breeds, identified a single deleterious candidate variant: a stop-gain mutation in the Protein O-Mannosyltransferase 2 gene (POMT2; Chr10:g.88499167C >T; POMT2 p.W334X). The POMT2 gene encodes an enzyme involved in the glycosylation of α-dystroglycan. This process is essential for maintaining the stability of muscle fibres and ensuring normal brain development. Notably, several deleterious missense variants in POMT2 have been associated with congenital muscular dystrophy and brain defects in humans, whereas complete knockout of the gene results in embryonic lethality in mice. If expressed, the mutant protein would be truncated by approximately 62% and would lack several essential functional domains. Large-scale genotyping of the POMT2 variant in 94,727 Normande cattle using the custom Illumina EuroGMD revealed an absence of homozygotes. The allele frequency was estimated to be 1.8%. Among 1,275,893 additional animals from 20 breeds, all were wild type except nine Holsteins, which had some degree of Normande ancestry. In conclusion, we report a novel recessive genetic defect in Normande cattle, primarily responsible for stillbirth and, to a lesser extent, reduced fertility. The identification of the likely causative variant, now included on the genomic evaluation array, will allow efficient monitoring and help prevent future at-risk matings.

Population genomics of 1,060 French dairy, beef, and local cattle breeds using whole-genome sequencing-derived SNP panel

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Abstract Cattle domestication, together with other evolutionary forces such as selection and demographic changes, has significantly shaped genome-wide genetic variation, contributing to a wide range of genetic diversity and giving rise to the modern cattle breeds. French cattle populations represent substantial genetic diversity, comprising approximately 40 recognized breeds with diverse production purposes and local adaptations. Given their remarkable phenotypic diversity, it is necessary to study how the genetic variation across French cattle breeds is structured, as it may reflect potential shared ancestry, historical demographic events, selection practices, and geographical adaptation. To better understand the genetic structure of French cattle breeds, we conducted, in this study, a population structure analysis by combining a Principal Component Analysis (PCA), Admixture, and pairwise Fst (fixation index). The analysis was performed on single-nucleotide polymorphisms (SNPs) obtained from whole-genome sequencing data of 1,060 individuals representing 21 French cattle breeds. As expected, we observed notable genetic divergence between Holstein and the other 20 cattle breeds, reflecting Holstein's distinct origin, breeding history, and strong selection for milk production traits. Meanwhile, within the remaining 20 breeds, we noticed significant evidence of genetic overlap, suggesting potential historical gene flow and shared ancestry. The SNP panel we have constructed provided a valuable understanding of the genetic variation and relationships between French cattle breeds. Moreover, it will serve as a critical foundational material for future research on selection signatures aimed at identifying genomic regions and variants under selection pressures, thereby improving targeted breeding strategies and contributing to an effective conservation program of local breeds. In conclusion, our study represents comprehensive information on population structure and potential historical events within the French cattle population. Our study will be valuable for future projects on selection signature analysis, and conservation and breeding programs aiming to enhance the sustainability of the agriculture industry.

Allele-specific methylation and parent-of-origin QTL mapping reveal potential epigenetic mechanisms underlying genomic imprinting and beef traits in Nelore cattle

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Parent-of-origin effects (POEs) occur when phenotypic variance depends on whether an allele was inherited from the sire or the dam. When a POE impacts gene expression patterns by silencing the allele derived from one specific parent, genomic imprinting occurs. Allele-specific methylation (ASM) can contribute to genomic imprinting and allele-specific expression (ASE). In this study, we investigated ASM and POEs in skeletal muscle of Nelore (Bos indicus) steers to uncover regulatory mechanisms underlying fatty acid composition, mineral concentration, and meat and carcass quality traits. Using reduced-representation bisulfite sequencing (RRBS) and sample-matched genotypes from 12 animals, we identified 8,074 ASM regions, including 269 that were consistently detected across all samples, indicating reproducible ASM signatures in bovine muscle. ASM events were not uniformly distributed but were predominantly enriched in promoters. We also detected loci with dense ASM events overlapping or near ASE SNPs previously reported in the same population, suggesting regulatory hotspots where genetic and epigenetic allelic mechanisms converge. ASM density increased toward chromosome ends, consistent with broader chromosomal regulatory contexts enriched for CpG islands. Integration with imprinting resources supported the biological relevance of our findings. ASM occurred within eleven genes previously reported as imprinted in cattle, including the IGF2R locus, imprinted in muscle. IGF2R showed multiple ASM regions (including promoter ASM regions with predicted transcription factor (TF) binding sites) and an intronic ASE SNP. Additionally, 111 ASM regions were assigned to genes previously reported to be monoallelically expressed, representing potential new candidates for imprinting in cattle. To quantify POEs at the phenotype level, we applied an omnibus test to perform a parent-of-origin GWAS and identified 1,817 PO-QTLs distributed across five clusters of correlated traits, with the strongest signal observed in polyunsaturated fatty acid-related phenotypes. Cluster-specific enrichments were biologically coherent for each trait cluster, and candidate loci showed predicted TF and miRNA binding effects. Overlap between ASM and PO-QTLs was limited (seven PO-QTLs overlapping ASM SNPs; ~0.38%), likely reflecting reduced informative SNP density in conserved ASM/imprinted regions and limited statistical power. Overall, the integration of our variants and ASE SNPs showed 600 variants overlapping previous ASE data from the same population, prioritizing epigenetically regulated loci for functional validation. Our findings advance understanding of regulatory variation affecting beef quality traits in Nelore cattle. Keywords: epigenetics, Bos indicus; beef.

Beyond SNPs: INDELs as functional drivers of feed efficiency in Nellore cattle

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Selection for feed efficiency (FE) is fundamental to the profitability, sustainability, andreduction of the environmental footprint of tropical beef cattle production systems.While SNP-based analyses have advanced, evidence remains limited regarding thecontribution expressed insertion-deletion variants (INDELs) to residual feed intake(RFI) variation, specifically in Nellore cattle and their functional interpretation throughbiological pathways. As structural variants that can alter protein-coding sequences andregulatory regions, INDELs represent a potentially important source of functionalvariation. The objective of this study was to identify functional INDELs associated withRFI in two RFI groups of Nellore cattle using RNA-Sequencing data. Transcriptomicdata from five tissues (liver, muscle, hypothalamus, pituitary, and adrenal glands) wereanalyzed from 18 Nellore bulls (9 high-RFI vs. 9 low-RFI). The INDELs were detectedusing CLC Genomics Workbench (Aarhus, Denmark) and its functional impact wasassessed using the Ensembl variant effect predictor (VEP) based on the ARS-UCD1.3assembly. Metabolic pathway enrichment analysis was conducted using the softwareDAVID. Results showed that expressed INDELs carried high-impact functionalconsequences mapping to multiple metabolic pathways. Among the 285 INDELsdetected, 155 were shared between groups, reflecting shared genetic variation. In thelow-RFI (efficient) group, 67 exclusive INDELs were identified within 106 genes.Among them, 7 INDELs showed amino acid changes (AAC), 5 INDELs with spliceeffects (SE), and 2 variants with both AAC and SE events. The VEP analysis prioritized8 variants as having high predicted functional consequences (frameshifts and stop-gained) and 5 with moderate impact. The high-RFI (inefficient) group harbored 63INDELs located within 103 genes, including 7 AACs, 4 SEs, 1 with both AAC and SEevent, and 9 high-impact transcripts. Pathway analysis of expressed INDELs revealed 8significantly enriched pathways in the low-RFI group and 10 in the high-RFI group (P <0.05). Specifically, the low-RFI group included phagosome and cytoskeleton in musclecells, whereas the high-RFI group involved HIF-1 and cAMP signaling. Biologically,pathways in low-RFI cattle focused on cellular recycling and structural integrity. Incontrast, high-RFI cattle prioritized energy-intensive signaling and immune alerts,suggesting a metabolic trade-off that diverts resources from production. These resultsdemonstrate that expressed INDELs carry a disproportionate functional effect,potentially altering key metabolic pathways associated with RFI. The higher frequency of prioritized high-impact INDELs in inefficient animals suggests a greater geneticburden affecting metabolic stability. Characterizing these structural variants identifiespromising targets for genomic selection to improve nutrient utilization in Nellore cattle,advancing the development of efficient herds aligned with sustainable beef productionsystems.

Ovine hypothalamic neurons under proteostatic pressure during leptin resistance

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KNDy neurons of the mediobasal hypothalamus constitute a key regulatory node integrating metabolic and endocrine signals controlling pulsatile gonadotropin-releasing hormone (GnRH) secretion and reproductive function. Proper functioning of these neurons depends on their ability to sense metabolic cues such as leptin and circulating nutrients. Metabolic disturbances associated with obesity and chronic lipid oversupply frequently lead to leptin resistance, disrupting hypothalamic signaling and contributing to impaired reproductive regulation. Increasing evidence suggests that metabolic stress may also affect cellular proteostasis by altering protein turnover, folding and degradation pathways essential for neuronal homeostasis. However, the molecular mechanisms linking metabolic stress with proteostatic regulation and intracellular signaling in hypothalamic neurons remain insufficiently characterized. The aim of this study was to identify proteomic and phosphoproteomic alterations associated with lipid-induced metabolic stress in ovine hypothalamic neurons. An immortalized ovine KNDy neuronal cell line (FRI273), derived from fetal sheep mediobasal hypothalamus and kindly provided by Dr. Patrick Chappell, was used as an in vitro model of hypothalamic neurons integrating metabolic and reproductive signals. Cells were cultured in high-glucose DMEM supplemented with 10% FBS and exposed to palmitate (5 mM, 24 h) to model lipid-induced metabolic stress associated with leptin resistance. Untreated cells served as negative controls and NEFA-free BSA was used as vehicle control. Quantitative TMTpro-16plex proteomic and phosphoproteomic analyses were performed using LC–MS/MS on an Orbitrap Exploris 480 mass spectrometer. Data were processed using MaxQuant and Perseus (v1.6.15.0) against the Ovis aries UniProt reference database. Proteomic profiling quantified 7,712 proteins, of which 1,123 were significantly altered in palmitate-treated neurons compared with controls (FDR < 0.05). Proteins involved in protein quality control and degradation pathways were markedly increased, including sequestosome-1 (SQSTM1; FC 4.31), GABARAPL2 (FC 5.39), PDIA6 (FC 1.55) and alpha-crystallin B chain (HSPB1; FC 1.65), indicating activation of autophagy-related and chaperone-mediated proteostasis mechanisms. Phosphoproteomic analysis identified 4,086 phosphosites, of which 739 showed significant changes (FDR < 0.05). Differential phosphorylation affected regulators of cytoskeletal organization and signal transduction, including vimentin (FC 2.69), microtubule-associated protein 1B (FC 1.45) and tubulin (FC 1.30). Altered phosphorylation was also observed in signaling regulators such as stathmin, PEA-15, 14-3-3 proteins and AKAP-associated signaling components, indicating remodeling of cytoskeletal dynamics and kinase-dependent signaling pathways. Collectively, these data indicate that lipid-induced metabolic stress linked to leptin resistance subjects ovine hypothalamic neurons to significant proteostatic strain, resulting in reconfiguration of phosphorylation-dependent signaling pathways. Coordinated alterations in protein turnover and signaling processes may represent adaptive mechanisms allowing hypothalamic neurons to maintain functional stability while integrating metabolic signals affecting neuroendocrine regulation of reproduction. This research was financially supported by the National Science Centre, Poland (Grant No. UMO-2023/51/B/NZ9/00651).

The in ovo application of synbiotics may influence the humoral immune response against homologous antigens—transgenerational insights

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In humans, the use of probiotics in patients with allergies suggests their potential role in inducing immune tolerance. The in ovo application of synbiotics on day 12 of egg incubation exposes the embryo's developing immune system to foreign antigens. This exposure may influence the processes of immune recognition and discrimination between self and non-self. This study aimed to evaluate whether single (F1 generation) or repeated (F1–F3 generations) in ovo administration of a synbiotic (PoultryStar® SolUS, DSM-Firmenich; hereafter referred to as PS), or synbiotic combined with choline, applied into the air chamber on day 12 of incubation, may modulate the humoral immune response recognition against surface antigens of the probiotic in three conscutive generations. Serum samples in the F1 generation were collected from seven 14-day-old chickens and seven 21-week-old birds from the following groups: control (C), SYNs (single in ovo synbiotic stimulation in F1), and SYNCHs (single in ovo synbiotic+choline stimulation in F1), all treated on day 12 of embryonic incubation. In the F2 and F3 generations, serum samples were obtained from seven 14-day-old chickens and seven 21-week-old birds from the following groups: C, SYNs, SYNr (repeated in ovo synbiotic stimulation across F1–F3), SYNCHs, and SYNCHr (repeated in ovo synbiotic + choline stimulation across F1–F3), all treated on day 12 of embryonic incubation. Serum IgG and IgM antibodies directed against outer membrane complex (OMC) antigens of the synbiotic preparation, harvested using Triton X-114 extraction, were determined by ELISA. IgG antibodies against SDS-PAGE separated antigens were determined in immunoblotting. The delayed-type hypersensitivity (DTH) reaction following intradermal injection of synbiotic antigens was measured in 42-day-old F3 chickens. In the serum of 14-day-old and 21-week-old F1 birds, the detection of OMC bands differed between control and treated animals in Western blotting. The sera of birds treated with synbiotic recognized a different spectrum of antigens than the group treated with symbiotic + choline. ELISA intensity of IgG and IgM serum antibody reactivity against harvested synbiotic antigens did not differ between control and treated groups within generations, although the mean values were markedly lower in treated than in control animals. Serum antibody reactivity decreased progressively across subsequent generations in both 14-day-old and 21-week-old birds. The broad spectrum of recognized antigens found in control and treated birds concurrently indicates the possible cross-reactivity between antigens solubilized from PS synbiotic and environmental bacteria antigens recognized by examined birds. The exposure of developing embryos to the PS antigens may modulate the development of humoral immunity against environmental antigens. The question arises whether the introduction of PS antigens/probiotic bacteria with potentially tolerogenic effects into the facility environment may be an important factor influencing not only treated but also control animals.
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Homozygous haplotype enrichment/depletion mapping identified a recessive variant in ALKBH8 causing rearing-loss in Brown Swiss cattle

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Most cattle breeds have a small effective population size and are therefore susceptible to the emergence of deleterious recessive traits. Such traits are typically investigated using phenotype-driven approaches that require clearly affected individuals; however, these strategies may fail to detect conditions with late onset or nonspecific clinical presentation, including immune, reproductive, or metabolic disorders. We aimed to discover cryptic recessive variants in Swiss dairy cattle affecting survival by exploiting population-scale genomic data together with extensive life-history records. Brown Swiss (BS) cattle, the second most popular dairy breed in Switzerland have experienced increasing inbreeding due to intensive selection and the widespread use of elite sires, facilitating the expression of recessive alleles. Life-history records from 67,912 genotyped BS animals were analyzed to identify cases of juvenile mortality (≤180 days) using carcass disposal records. Recessive haplotypes associated with juvenile death were detected using imputed genotypes for more than 600K genome-wide SNPs and Homozygous Haplotype Enrichment/Depletion mapping. Expectations for haplotype homozygosity in case and control groups were estimated based on genotyped parents and grandparents. Haplotypes showing ≥25% enrichment of homozygosity in cases and ≤−25% enrichment in controls were considered candidate associations. Selected homozygous animals underwent long-read whole-genome sequencing (WGS), followed by detailed clinicopathological evaluation. We identified a haplotype on chromosome 15 associated with increased juvenile mortality in the homozygous state. The allele frequency of the linked haplotype in BS was estimated to be 4%. Haplotype carrier prediction was performed on a population level using available genotyping data from the Swiss genomic selection. This allowed the identification of living young homozygous haplotype carriers. Five homozygous animals consistently exhibited failure to thrive characterized by recurrent respiratory disease and diarrhea, reduced body condition, and a rough hair coat. Marked brown discoloration of the incisors was observed in all affected animals. Hematological analyses revealed hypoglobulinemia. Intestinal coccidiosis was detected in all examined calves. Pathology revealed bronchopneumonia, accompanied by eosinophilic enterocolitis in the two examined cases. These findings indicate failure to thrive and immunodeficiency in homozygotes. Long-read WGS of two homozygous calves revealed a single structural variant consisting of a 10.4-kb ERV_classII LTR insertion in exon 3 of ALKBH8 representing a loss-of-function similar to the APOB insertion found in cholesterol deficiency affected Holstein cattle. Our results provide strong evidence for a previously unrecognized hereditary rearing-loss disorder in BS cattle and illustrate how integrating genomic resources with life-trajectory data, clinical research and molecular genetics enables the detection of cryptic recessive conditions. Indirect and direct carrier detection enables targeted breeding strategies essential to prevent homozygous animals reducing rearing losses and improve animal welfare.

Unravelling signatures of Red Holstein introgression and its haplotype structure in Fleckvieh cattle

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Between the 1970s and the 1990s, Red Holstein (RH) sires were crossbred into the German Fleckvieh (FV) population to enhance milk yield and udder traits while maintaining breed-specific characteristics of the dual purpose FV cattle. Through the use of RH bulls and their male offspring, RH-derived chromosomes and chromosomal fragments were incorporated into the FV genome and subsequently selected positively or negatively according to the breeding goal. In this context, it is of interest which genomic regions exhibit above-average RH proportions and whether certain regions show significantly lower RH contributions than expected. Therefore, the aim of this study was to quantify the genome-wide distribution of RH introgression in FV cattle, and to characterize the underlying haplotype structure and diversity of the introgression segments. For this purpose, a total of 58,849 haplotyped and imputed genotypes, comprising 50 pure FV animals (born before Dec. 31, 1985), 65 pure RH animals (born before Dec. 31, 1995), and 58,734 FV animals with an initially unknown proportion of RH ancestry (born after Jan. 1, 2010) were analyzed. Identity-by-descent (IBD) segments of RH origin were detected from phased genotype data using the software hap-IBD (Zhou et al., 2020). Based on these segments, individual genomic RH proportions were estimated and a genome-wide map of RH haplotype frequencies was constructed to identify regions deviating from the expected RH contribution. Across the 58,734 FV animals, the mean genomic RH proportion was 4.39% (SD = 2.35%, SEM = 0.01%), ranging from 0% to 39.08% after excluding 136 non-representative animals. Considering the average observed RH proportion in our FV population of 4.39%, we identified chromosomal regions with significantly higher frequencies of RH haplotypes than expected (e.g. BTA 8: 54.2-112.3 Mb, up to 20.38% RH haplotypes per position; BTA 20: 58.3-64.2 Mb, up to 12.71% RH haplotypes per position; BTA 25: 21.4-38.7 Mb, up to 17.56% RH haplotypes per position). At peak positions, introgression can be largely explained by a limited number of donor haplotypes: on BTA 8, more than 80% of the introgressed haplotypes could be traced back to the RH sires JAMES RED, TOPPER and SKAKERAK, whereas on BTA 20 and 25, a single haplotype originating from JAMES RED accounted for 71% and 61% of RH-derived segments, respectively. In addition, window-based analyses were conducted to identify regions of reduced haplotype diversity, providing a more refined understanding of the introgression pattern. Ongoing analyses aim to investigate introgression dynamics at the level of individual RH sires to further elucidate founder-specific contributions to the FV genome. Overall, this approach may also be valuable for investigating introgression patterns in other cattle breeds or animal species with known crossbreeding history.

How farms are impacted and deal with climate change in grassland systems: the case of Occitanie dairy sheep farms in southern France

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In France, sheep milk is mainly produced in 3 production areas located in dry and poor disadvantaged mountain regions of the south of the country. Among these areas, Occitanie is the largest one and accounts for 74% of the national production, with flocks of Lacaune dairy sheep breed. Because of the Roquefort protected designation of origin specifications and varied local soil and climate conditions, dairy sheep farms mainly rely on cultivated grasslands and spontaneous vegetation. Lacaune ewes receive diet based on fresh and preserved grass. In this mid-mountain region, where Mediterranean and semi-continental climates coexist, climate change is mainly manifested by inconsistent annual conditions, particularly in recent years, with alternating cold and dry year (2021), hot and dry year (2022), and very wet years (2023 and 2024). Not all farms have the same response to these changes. Some have implemented adaptation practices making them more resilient, while others, which are more robust, suffer less from the consequences of this high climate variability. Nevertheless, some farms remain vulnerable and must therefore deal with the consequences of climate change. It may have direct impacts on the health and performances of the dairy ewes, but also on the main source of animal feed, grassland, in both terms of yields and forage quality, and utilization of pastures. This work therefore aimed to characterize the effects of climate change on dairy sheep farms in Occitanie, with a particular focus on farm technical-economic results, including milk composition and production, feed autonomy, and sheep health. Monitoring meteorological data and the results of 30 dairy sheep farms in the region over the period 2020–2024 allowed to identify farms that are resilient and/or robust in response to climate change from those being more vulnerable. At the same time, a characterization of adaptation practices or trade-offs implemented by farmers to reduce their vulnerability in terms of feed autonomy, herd health, and variations in milk production and quality, was performed. This work will identify levers that can be used to make dairy sheep farms in the Occitanie region less vulnerable to climate change.

Grassland Yield and Quality in Relation to Farming System and Intensity

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Slovenia is a land of permanent grasslands, which represents an important source of forage on cattle farms. High-quality voluminous forage represents an important part of the feed ration on every cattle farm and, consequently, the basis for profitable ruminant husbandry. The introduction of a reliable, user-friendly and affordable method for assessing the increase in the mass of grass on meadows and pastures is necessary from the point of view of the effectiveness of the permanent grassland management. In the past, a lot of efforts has been invested in development of methods for indirect and non-invasive assessment of the growth of grass. The most effective and precise method is to take measurements with the help of a digital platemeter. This measures the height in relation to the density of compressed grass, which directly depends on the amount of dry matter (kg DM/ha). Differences in grass height, density, botanical composition, season and management method can thus increase the error in the prediction of grassland mass estimation. Assessment of grass mass must be carried out weekly during the growing season. The obtained results later serve us to calculate the daily amount of the available amount of grass or pasture. Accurate and quick assessment of the available grass or pasture on the farm helps in making strategic decisions from the point of view of permanent grassland and improvement of grazing management. As part of the pilot EIP project entitled "Feed ratio and biodiversity under climate change conditions – the example of a cattle farm", we carried out measurements of grass mass yields on three pilot cattle farms during the growing season on a weekly basis, with the help of a Jenquip EC20 digital platemeter.

Seasonal variation of water-soluble carbohydrates of temporary grassland in a Mediterranean agroforestry system

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In 2017, a Long-Term Experiment (LTE) based on a 40-ha agroforestry trial site was established in Pisa (Tuscany, Italy) to study the diversification of farming systems. The investigated system is based on a seven-year crop rotation consisting of durum wheat, sorghum, faba bean, and four years of mixed temporary grassland intercropped with rows of poplar trees spacing 30 m between and 10 in rows. In 2025, a field campaign was carried out to evaluate the effect of tree presence on the water-soluble carbohydrate (WSC) of herbage mass obtained from a temporary grassland during its first year of establishment. WSC are an important energy source for grazing animals, but high concentrations have been linked to pasture-associated laminitis and acidosis. The experimental design aimed to mimic grazing activity according to biomass accumulation. Samples were collected at five different distances from the tree row according to a replicated transect design, to evaluate yield and nutritional characteristics of the herbage. The temporary grassland was sown in October 2024, and the first harvest was carried out in April 2025 to ensure adequate root establishment. Following harvests were performed when herbage reached an average height of about 0.12 m, measured using a plate grassmeter and herbage dry matter (DM) production was determined. Samples were analysed for botanical (grasses, legumes, and other species) and chemical composition, with a focus on WSC. The average DM production over the harvest was 2.78 ± 1.96 Mg ha^{-1} and varied significantly during the season, consistent with Mediterranean climatic patterns. The first harvest showed higher biomass production, whereas the second and third harvests showed an average reduction of about 71% compared with the first harvest. The botanical composition consisted of 75.32 % ± 24.12 grasses, 4.45 % ± 13.87 legumes, and 20.23 % ± 23.48 other species throughout the entire period. A significant tree effect on DM production was observed only at the second harvest, with higher yields near trees and a greater proportion of grasses in that zone. However, the tree presence did not significantly affect WSC content. WSC averaged 75 ± 25 g kg^{-1} DM, consistent with literature values, but decreased from 100.03 g kg^{-1} DM, at the first harvest, to 72.67 and 53.57 g kg^{-1} DM at the second and third harvests, respectively.The same trend was observed for glucose and fructose, which decreased by one quarter and one third, respectively, leading to a marked reduction in herbage WSC content. Fructans concentrations remained stable, averaging 23.85 ± 4.98 g kg^{-1}DM. However, considering seasonal trends in total WSC and its individual components, the relative contribution of fructans to total WSC increased during the season. Sucrose and raffinose showed low concentrations compared with the other WSC components, averaging 1.31 ± 0.95 and 1.40 ± 1.29 g kg^{-1} DM, respectively. Raffinose did not significantly vary among harvests, whereas sucrose approximately doubled its concentration from the first to the last harvest. In conclusion, seasonal trends showed decreasing WSC, even if fructans content was stable in herbage DM and increased its concentration in the WSC in later harvests. These findings provide original knowledge of WSC dynamics in Mediterranean temporary grasslands in agroforestry systems.

Rewetting a major Dutch peat region: assessing protein production under environmental policy targets

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The majority of peatlands in the European Union are drained to increase agricultural production, resulting in greenhouse gas emissions, soil subsidence and biodiversity loss. Therefore, restoration of peatlands plays a major role in the transition towards a more sustainable food system. The European Green Deal, through the Nature Restoration Regulation, aims to recover the contribution of agricultural peatlands to ecosystem services and biodiversity conservation. Proposed measures include rewetting drained peat soils (i.e. increasing the groundwater level) and reducing nitrogen inputs. In the light of these policy objectives, using a case study of the Netherlands, where peat soils are predominantly used as permanent pasture for dairy production, we explore how peatlands can contribute to protein production while meeting environmental policy targets. We use a food system optimization model, FOODSOM, to maximize protein output in several scenarios, simulating increasingly ambitious policy targets. Scenarios ranged from maximizing protein production without policy constraints, to letting 10% of land aside, partial rewetting of peat areas and full rewetting of peat areas. Preliminary results show that across scenarios, total protein production reduced by 17%, with a 25% reduction in milk-based protein. This coincides with the observed 25% reduction in the number of dairy cows, which was accompanied by a shift to more low-productive dairy cows. Total greenhouse gas emissions from the food system reduced on average by 37% across scenarios, with a 47% reduction in emissions originating from peat soils. In a next step, the impact of grassland diversification (i.e. the introduction of herb-rich grasslands) on the protein production potential of the region will be further explored to optimize the performance of grasslands on peat soils. Overall, our results suggest that there is room to keep peat soils productive while adhering to environmental targets. Yet, substantial changes in farming systems and business models are required.

Tree thinning effects on animal performance in tropical silvopastoral system

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Silvopastoral systems (SPS), which integrate pasture, trees, and animals in the same area, represent a sustainable alternative for pasture-based livestock production. One of the main challenges of these systems is maintaining the productive potential of pastures and, consequently, livestock production. This study aimed to evaluate the effect of tree thinning in a silvopastoral system on animal production compared with full-sun pasture-based production systems. The study was carried out at Embrapa Pecuária Sudeste, in São Carlos, São Paulo State, Brazil (21°57′ S, 47°50′ W; 860 m a.m.s.l.), from 2020 to 2023. The evaluated systems were: an extensive system (EXT), consisting of Urochloa decumbens cv. Basilisk pasture under continuous stocking, without liming or fertilization; an intensive system (INT), consisting of U. brizantha (Hochst. ex A. Rich.) Stapf cv. BRS Piatã pasture managed under rotational stocking; and a silvopastoral system (SPS), consisting of U. brizantha Stapf cv. BRS Piatã pasture managed under rotational stocking, shaded by rows of eucalyptus (Eucalyptus urograndis, clone GG100). The trees were planted in 2011 at a spacing of 15 × 2 m (333 trees ha^{-1}), oriented approximately east–west, thinned in 2016 to 15 × 4 m (166 trees ha^{-1}), and thinned again in 2019 to 30 × 4 m (83 trees ha^{-1}). The INT and SPS were consisted of four 3-ha areas, subdivided into six 0.5-ha paddocks, managed under rotational grazing with liming and fertilization (132 kg N ha^{-1} $year^{-1}$) and grazed by Nellore and Canchim (3/8 Zebu × 5/8 Charolais) beef steers, totaling 16 animals per system. The EXT system consisted of two 3-ha areas, without subdivision, stocked with a total of eight animals. Photosynthetically active radiation (PAR) transmission in the SPS, as well as animal performance and productivity expressed as average daily gain (ADG, kg day^{-1}), stocking rate (SR, animal units ha^{-1}), and live weight gain per ha (LWGha, kg ha^{-1} $year^{-1}$), were evaluated. Data were subjected to analysis of variance using PROC MIXED of SAS software, and means were compared using Fisher's test ($p < 0.05$). PAR transmission in SPS ranged from 71% in Year 1 to 60% in Year 3. ADG did not differ among production systems and was higher in Year 3 (0.561 kg day^{-1}) compared with Years 1 (0.451 kg day^{-1}) and 2 (0.406 kg day^{-1}), which did not differ from each other. There was a significant interaction between systems and experimental years for SR and LWGha. Stocking rates were similar between INT and SPS in Years 1 (~2.14 AU ha^{-1}) and 2 (~2.40 AU ha^{-1}), which were higher than EXT (1.34 and 1.33 AU ha^{-1}, respectively). In Year 3, SR was highest in INT (2.57 AU ha^{-1}), followed by SPS (2.14 AU ha^{-1}) and EXT (1.33 AU ha^{-1}). LWGha was similar between INT and SPS in Years 1 (~360.3 kg ha^{-1} $year^{-1}$) and 3 (~439.1 kg ha^{-1} $year^{-1}$), which were higher than EXT (130.5 and 255.9 kg ha^{-1} $year^{-1}$, respectively) . In Year 2, LW was highest in INT (728.4 kg ha^{-1} $year^{-1}$), followed by SPS (613.4 kg ha^{-1} $year^{-1}$) and EXT (285.7 kg ha^{-1} $year^{-1}$). These results demonstrate that tree thinning in tropical silvopastoral systems enables high animal productivity, comparable to that in well-managed pasture systems without trees.
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Role of Grasslands in Enhancing Sustainability and Product Quality in Cattle Farming. Insights from the INTAQT project.

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The EU cattle sector relies on a wide variety of farming systems. The EU Horizon 2020 INTAQT project (Grant Agreement No. 101000250) has sought to understand how the diversity of farming systems and practices influences the quality and authenticity of cattle and chicken products, as well as the sustainability of their production. A key concept of the project is "One Quality", which considers intrinsic product quality alongside broader issues such as environmental impact, feed-food competition, and animal welfare. For cattle, the project is based on a network of 131 beef and dairy farms spanning eight European countries and covering a wide range of systems, from the most extensive to the most intensive.We defined levels of intensification based on inputs relating to animals (e.g. concentrates) and land (e.g. fertilisers). For both beef and dairy farms, intensification improves productivity and the efficiency of total feed and land use, but the benefits tend to plateau in high-input systems. However, when considering only human-edible protein in feed and arable land use, intensification exacerbates feed-food competition, with grass-based beef and dairy systems being net human-edible protein producers. Intensification also reduces the provision of regulating ecosystem services by farms, which balance the well-documented higher GHG emissions of extensive farming when expressed per kg of product. Intensification did not affect the animal welfare indicators recorded on-farm, but it was associated to a reduced outdoor access during the life of cattle. None of the chemical contaminants analysed (heavy metals, myco- and plant-toxins, dioxins, PCBs, PFAS) in beef liver and milk exceeded the official safety limits. In our farm network, access to pasture in extensive farms did not result in increased product contamination. However, the important amount of maize silage and concentrates used on intensive farms led to a slight increase in aflatoxin M1 in milk, although the upper limits were never exceeded.The project confirmed the well-documented influence of feeding fresh grass on the sensory properties of cheese and revealed that grass-based farming systems produce yellower and creamier UHT milk with a stronger flavour than UHT milk from maize-based systems. However, these grass-based cheeses and UHT milks were not necessarily more appreciated by naive consumers in blind tests. Similarly, the eating quality of beef striploin, as evaluated by naive consumers, was not related to the intensity gradient of the farming systems.Finally, high variability was observed for most nutritional quality traits both in beef and milk, which should be considered by nutritionists. Some quality traits were strongly related to farm intensification, which reduced for instance the concentration of beneficial fatty acids in beef and milks. Intensification also reduced the levels of vitamins E and B2 in milk, while increasing the levels of vitamins B5 and P. The most significant changes were primarily associated with the proportion of grass in the diet and the age at slaughter (for beef).Overall, this project reveals the central role played by grasslands in the « One Quality » of cattle products, whether positive or negative according to the quality traits considered. However, it should be noted that high variability is generally observed within each farm type for most quality traits, indicating potential for improvement. www.h2020-intaqt.eu

Living with wolves? Cross-country differences in perceived predator constraints to agroecological grazing

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Agroecological grazing is not only shaped by ecological conditions but also by the social and institutional context. Understanding constraints perceived by future farmers is essential for maintaining and improving grassland-based livestock systems. Within the Grazing4AgroEcology project, a survey conducted in 2023 among young livestock farmers and agricultural students (≤40 years) in eight European countries explored perceived barriers to grazing. Predators such as wolves were frequently mentioned among the top five barriers to grazing, but strong regional differences were observed. The share of respondents selecting predators in their top five barriers to grazing ranged from 7% in Ireland to 78% in South Tyrol (Italy), with relatively high values in Germany (64%) and Sweden (53%), and moderate values in the Netherlands (19%), France (22%), Romania (22%), Portugal (23%) and Sardinia (Italy) (24%). These perceptions did not align with actual wolf densities. Countries with long-standing high wolf populations, such as Romania, showed relatively moderate concern. In contrast, countries where wolves have only recently returned or expanded, such as Germany, reported higher perceived barriers despite lower ecological densities. This suggests that predator presence becomes constraining particularly in "return contexts", where grazing systems, advisory structures and governance arrangements are still adapting to the change. This indicates that perceived predator pressure is shaped not only by the number of predators present but also by the pace of change and experience with coexistence. Where interaction with wildlife has historically been part of livestock production, grazing systems are perceived as more resilient to predator presence. In regions experiencing recent predator return, uncertainty, institutional adaptation gaps and limited practical experience may affect grazing decisions and continuity. This pattern is relevant for countries such as the Netherlands, where wolf presence is a recent but rapidly developing reality. After more than a century of absence, the first individual wolf was recorded in the Netherlands in 2015, followed by re-establishment of territorial wolves from 2018 onwards. The number of wolf packs increased from one between 2019–2021 to eleven in 2024 with an estimated total number of 100-150 wolves. Over the same period, confirmed livestock attacks rose from 426 cases in 2023 to 795 in 2024, resulting in over 2,400 killed animals. While full monitoring results for 2025 are not yet available, preliminary data indicate continued population growth alongside persistently high levels of livestock damage. For comparison, Romania, which is about 6 times the size of the Netherlands, is home to one of the largest wolf populations in Europe, with estimates often ranging between 2,500 and 3,000 individuals. There were reported livestock losses that can be managed by local shepherds using compensation schemes. Improving the sustainability of grassland-based systems in return contexts requires not only technical mitigation measures but also coordinated governance responses and knowledge exchange. Supporting learning processes, advisory capacity and collaborative design of grazing strategies may enable workable coexistence between livestock and wildlife. In regions undergoing predator return, grazing resilience will depend on the adaptive capacity for coexistence rather than predator density alone.

Beyond predation Hotspots: Livestock resilience during 26 years of wolf recolonization in the French Alps

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Mountain livestock systems operate within complex socio-ecological contexts and face increasing challenges. In the European Alps, mountain livestock systems rely on seasonal grazing on large alpine pastures, where interactions between livestock and wildlife can occur. Among wildlife species, the wolf (Canis lupus) has received increasing attention in livestock systems during recent decades following the recolonization of Alpine territories. This study analyzed the dynamics of spatial patterns of geolocated wolf predation on cattle and ovicaprids between 1998 and 2024 in the department of Savoie (French Alps), a mountainous territory characterized by extensive pastoral activity and strong altitudinal gradients. This study aimed to explore possible relationships between wolf predation and landscape composition, livestock distribution, and wildlife abundance. The analysis was conducted at the scale of inter-municipal administrative units (EPCI), which represent relevant territorial entities for agricultural organization and local governance. Each spatial unit was characterized in terms of landscape composition in relation to land cover (number of patches, average patch area, average Euclidean distance among patches, cover percentage), livestock system densities (dairy cattle, beef cattle, sheep and goats during 2000, 2010, and 2020), wildlife presence (wild ungulates from the official census between 1998 and 2024) and wolf predation (7 347 events resulting in 19 917 livestock losses between 1998 and 2024). Spatial analyses were performed to explore the distribution and concentration of predation events. Kernel Density Estimation (KDE) was used to identify areas of higher predation intensity, while Emerging Hot Spot Analysis (EHSA) was applied to examine spatio-temporal trends in predation events over the study period. Preliminary results revealed that ovicaprid predation was predominant and increased significantly over the years, with a peak during summer and heterogeneous spatial patterns characterized by persistent hotspots throughout the study period. Some areas showed increasing predation events, coinciding with and located close to the first colonized areas, while others exhibited decreasing trends, and still others showed no defined trends. The areas with the highest wolf predation were characterized by high ovicaprid density, low forest fragmentation, and abundant grassland-sparse vegetation patches. No relationship with wild ungulate populations was detected. Interestingly, the areas with increasing wolf predation also showed increasing livestock density, especially for ovicaprids, and only marginal decreases in cattle density, suggesting resilience of local livestock systems. This study contributes to improving knowledge about large carnivore-livestock interactions through the characterization of predation distribution trends in mountain contexts. This approach provides a spatial framework for analyzing predation patterns, laying the groundwork for assessing predation risk exposure and supporting local stakeholders in improving local strategies and prevention measures.

Seven New Rules for Worm Control In Sheep

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Sustainable Control of Parasites in Sheep (SCOPS) recommendations have provided clear guidance on resistance prevention for over 20 years, yet adoption remains low. Survey data (n=16,000, 2022) show 83% of Irish sheep farmers have never tested drench efficacy, 43% never calibrate dosing equipment, and 52% do not routinely quarantine, with adoption lower in cattle. In New Zealand, triple active resistance increased from 7% to 40% of tested farms (2020-2024), demonstrating how quickly efficacy can deteriorate. Further, climate change is increasing parasite challenge on farm. The objective was to identify practices used by farmers and programmes that have successfully maintained or restored drug efficacy. A mixed methods study (2023-2025) reviewed 217 publications and conducted semi-structured interviews (n=144) with parasitologists, veterinarians, breeders and farmers across five continents, purposively sampled across high-resistance (Australia, New Zealand), moderate-resistance (Ireland, UK) and low-resistance (Sweden) regions. Qualitative data was analysed using thematic analysis. Thematic analysis identified seven common practices among successful farmers and programmes: Start with the basics: Quarantine, efficacy testing and gun calibration are foundational, yet survey data showed most farms are not implementing these.Don't put all your eggs in the FEC basket: Knowledge transfer programme managers (n=4) recommended combining FEC with stockmanship, growth rate and pasture risk assessment rather than relying on FEC thresholds alone.The training should be hard so the game is easy: Breeders selecting for worm tolerance in Australia and New Zealand (n=9) reported halving treatment frequency within 5-7 years without compromising growth.The majority of lambs, a minority of ewes; almost never all: Adult ewes are generally accepted to have acquired immunity and not require routine treatment. However, survey data showed only 13% of farmers never treat ewes, and of those who do, 97% treat all animals. Published trial data support targeted selective treatment (TST). Blanket monthly treatment reduced ivermectin efficacy from 95% to 60% over five years whereas TST maintained efficacy at 87% with equivalent lamb growth and 45% less anthelmintic (Kenyon et al., 2013).Go beyond drugs, drugs and more drugs: Avoidance of parasites outperforms treatment. Published data showed unparasitised lambs had 50% higher ADG than parasitised lambs treated every 21 days. Co-grazing with cattle reduced parasite challenge by 40-60%.Shoot 'em, stab 'em, drown 'em: Multi-active combination treatment is standard practice in Australia and New Zealand. Modelling data indicates combinations extended time to drug failure from 87 to 300 treatments versus sequential use. A 7-year study (n=105 US farms) showed herbicide resistance developed 83 times slower with combination treatment. In human medicine, combination therapy for HIV, TB and malaria halved deaths from 4.8 to 2.5 million (2004-2024). Despite this evidence, combinations are unutilised in Europe.As much as necessary, as little as possible, but not zero: Parasitised lambs produce 33% higher methane emissions. Among UK organic farmers (n=226), only 20% achieved zero ewe treatments. As such, zero treatment is not a realistic target.Seven practices were identified among farmers and programmes that have maintained or restored drug efficacy. Analysis of knowledge transfer programmes (n=4) identified facilitator-led workshops, peer-to-peer farm visits, annual efficacy testing and investment in handling infrastructure as critical for adoption. These elements are being tested through the PARASCOPE EIP Project in Ireland (2026).

Effects of previous feed intensity on level of compensatory growth in growing cattle on semi-natural grasslands

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Semi-natural grasslands are habitat types characterized by substantial biodiversity and they are therefore of high conservational value. Continued livestock grazing over years is required for the values to maintain, but achieving high weight gains of growing cattle grazing semi-natural grasslands can be challenging. Previous research has identified the phenomenon of compensatory growth, which implies a negative correlation between feed intensity during an indoor period and weight gain during the subsequent grazing period. Compensatory growth is thought to be caused by increased appetite in combination with larger digestive tract in proportion to body mass and lower maintenance requirements in previously restrictively fed animals compared to more intensively fed animals. In addition to dietary composition and intensity level, intrinsic properties of the animal, such as gender and breed, or a natural variation over the year due to changes in metabolic rate or hormonal status can affect to what extent compensatory growth occurs. The aim of this study was to investigate the effect of previous indoor feed intensity on weight gain in growing cattle grazing semi-natural grasslands, i.e., compensatory growth. Data from growing cattle reared at SLU Götala Beef and Lamb Research Centre, south-western Sweden, in 36 trials from 1998 to 2025 was used. Average daily live weight gain (ADG) of 849 growing steers, heifers and bulls of dairy, beef, and dairy × beef breeds during their first indoor period after weaning (IP) and following grazing period (GP) was compiled. Diets during IP consisted of mainly grass-clover silage (50% – 100% of diet) in combination with grain and for young animals also protein feeds. During GP, the animals grazed heterogenous semi-natural grasslands with dry, mesic and wet areas as well as both open meadow and areas with scattered trees and shrubbery. For a preliminary comparison between levels of feed intensities, a sub-sample of 356 animals from seven trials, each with two indoor feed intensities (LOW and HIGH), was selected. The length of IP and GP for all animals was 184 ± 36 days (mean ± SD) and 168 ± 22 days, respectively, and the length of IP and GP for animals in the sub-sample was 180 ± 41 days and 155 ± 19 days, respectively. Across all animals, ADG was 0.88 ± 0.28 kg for IP and 0.56 ± 0.29 kg for GP. For animals in the subsample, ADG was 0.99 ± 0.26 kg for IP and 0.47 ± 0.31 kg for GP. When the subsample was split into feed intensities, a numerically higher ADG was observed during IP for the HIGH animals, 1.07 ± 0.20 kg/day, compared to the LOW animals, 0.9 ± 0.28 kg/day. During GP the opposite was observed, were the LOW animals expressed a numerically higher ADG, 0.57 ± 0.20 kg/day, than the HIGH animals, 0.37 ± 0.36 kg. Hence, the lower ADG during IP for the LOW animals was partly offset by their higher ADG during GP, where they managed to compensate 43% of the lost weight gain, i.e., 79 kg of 177 kg. A shorter GP (155 days) vs. IP (180 days) might partially explain why a higher recovery was not observed during GP. These findings indicate that the LOW animals experienced compensatory growth, which is consistent with previous research. More detailed results on the full data set will be available once the statistical analyses have been completed.

Body reserves dynamics of dual-purpose crossbred (Bos taurus × Bos indicus) cattle across successive production cycles under contrasting semi-extensive grazing farms in Cuba

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Future livestock farming systems (LFS) must be adapted to challenges imposed by climate change (CC), which are particularly important in low-input tropical LFS, mainly those based on grass and forage resources. The strategic and efficient use of the two complementary but antagonist phases of body reserves (BR) in ruminants [i.e., mobilization (BRM) and accretion (BRA)], plays a crucial role in the contribution of the animal component to the overall resilience of the LFS, when facing feed scarcity and fluctuations in nutritive value over time. Therefore, exploring the factors affecting BR dynamics (BRD) and the inherent intra-herd variability becomes a research priority in the current context, which has been mainly worked in temperate latitudes. The aim of this work was to characterize the BRD of tropical cows across successive production cycles reared under contrasting semi-extensive grazing LFS in Camagüey (Cuba; 21°N-77°W). Dual-purpose crossbred Bos taurus × Bos indicus cows (n=109), belonging to eight farms randomly selected in the region, were individually and longitudinally monitored to characterize their BRD over two consecutive years (April 2024-2026). The BR biomarkers assessed were: two zootechnical parameters (BW, body weight; BCS, body condition score), five metabolites (NEFA, non-esterified fatty acids; BHB, β-hydroxybutyrate; URE, urea; TGL, triglycerides; GLU, glucose) and the metabolic hormone T3 (triiodothyronine). Reproductive events were recorded and cows were phenotyped during eight key physiological stages (PhySt; i.e., early, mid, and late pregnancy; early, mid and late lactation; and dry period or maintenance). Relevant environmental and biological factors influencing BR traits were evaluated by performing analyses of variance (ANOVA) using the linear mixed model package (lmerTest) of the R Software (R Core Team, 2026; version 4.5.2). The cow was considered as random effect to take into account repeated measurements on the same animal over time. The farm (i.e., and their associated management practices), the climatic season (dry or rainy), the parity (primiparous or multiparous), the cow's PhySt, and their first-order and three-way interactions were considered as fixed effects. The BRD was strongly influenced by the farm management practices (mainly feeding-nutrition and reproduction). Unexpectedly, non-significant isolated effect of the year season was consistently observed on the BRD, which is in agreement with the complexity of the tropical research setting evaluated here (i.e., commercial LFSs without heat detection, calving synchronization or diet supplementation). The relevance of physiological effects (PAR, PhySt) was confirmed as the key modulating factors driving BRD, with BRM occurring from late pregnancy to early lactation, and BRA from mid-lactation to mid-pregnancy. These are the first results obtained on this subject under Cuban and Latin-American conditions. We provide relevant insights that allow a better understanding to decrypt the mechanism responsible of BRD in tropical cows, as well as their adaptive capacities in the current CC context. Future research efforts are warranted, considering the potential for leveraging this complex trait to establish adaptive farming practices and strengthen the resilience of semi-extensive pasture-based LFS in challenging tropical conditions.

Forage quality of rewetted peat grasslands: a preliminary evaluation

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Rewetting of peat grasslands aims to reduce greenhouse gas emissions; however, its effects on fodder productivity and harvestability remain unclear. To evaluate potential forage quality, samples were collected before each planned cut from one rewetted meadow (3.6 ha; rewetted two years ago) and one unrewetted control meadow (4.2 ha). Both meadows have been managed extensively in recent years, with up to two and three cuts per year, respectively. Samples were taken from up to three predefined areas per meadow, dried at 40 °C, and subjected to an extended Weender analysis. The forage is typically used for feeding beef cattle (silage or hay) and horses (grass cobs). Statistical analyses were performed in SAS (PROC MIXED), with meadow as a fixed effect and cutting date nested within meadow as a random effect. Due to persistently wet conditions during the harvest season, only one cut could be obtained from the rewetted meadow, carried out over three dates (July 18 to September 4). Not all harvested material could always be collected. The first cut of the control meadow was also conducted over three dates within two weeks (starting July 4), whereas subsequent cuts were harvested on single dates (last cut on October 19). No significant differences ($P > 0.05$) were observed between meadows for the evaluated parameters. Metabolizable energy for ruminants (mean ± SD) was 9.67 ± 0.06 and 10.32 ± 0.73 MJ/kg DM, crude protein was 79.42 ± 5.88 and 126.11 ± 49.48 g/kg DM, utilizable protein was 117.89 ± 0.54 and 132.00 ± 15.82 g/kg DM, ash-free neutral detergent fibre was 616.94 ± 4.70 and 499.00 ± 129.95 g/kg DM, and enzymatically soluble organic substance was 468.00 ± 32.05 and 629.33 ± 104.04 g/kg DM in forage from the rewetted and control meadow, respectively. Higher variability of the evaluated forage quality parameters was observed across cuts in the control meadow, consistent with harvest timing and regrowth effects. In contrast, variability among cutting dates was low in the rewetted meadow despite the prolonged harvest period, likely due to the simultaneous presence of senescent and newly emerging biomass. In conclusion, forage quality of the rewetted meadow was comparable to that of the control meadow. Overall, potential forage quality, based on pre-harvest and oven-dried samples, remained within a range considered adequate for beef cattle production, especially when combined with higher-quality forages or maize silage. Further sampling will be conducted to assess the long-term development of forage quality.

Evaluations of selected effects of feeding crushed grain to suckler cow-calves on the animals' fecal indices

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With increasing extensification of grazing areas and changing climatic conditions, a significant deterioration in the energy and nutrient content of pasture forage can be expected in a few years. Suckler cows can compensate for this to some extent by building up and breaking down their body reserves, which is not possible with growing calves. This would reduce the growth performance of the calves and thus also the weaning weight, which, however, has a certain significance for the economy of the process. Against this background, supplementary feeding of the calves with farm energy feeds such as grain can be discussed. The suckler cows calved in the period from the beginning of April to the end of May 2024, which is why 01.05.2024 was assumed to be the middle day of calving. As calves feed mainly on milk in the first 90 days of life (SCHOLZ, 2002), supplementary feeding was started on 01.08.2024. For this purpose, an automatic feeder with a storage container including a calf slop was used, which was placed as close as possible to the water wagon and the containers for the mineral feed. In order to be able to describe the effect of the supplementary feeding, pool faecal samples were analyzed 4 weeks before the start in order to have a baseline value. In the last week before the supplementary feeding, samples of the calves were collected intensively on 3 days and then also analyzed as pool samples. With the start of supplementary feeding, faecal samples were then repeatedly collected on a random day of the week to describe the key figures in the faeces. Dry matter and starch (enzymatic), nitrogen and crude ash were analyzed in the faecal samples. The digestibility of organic matter (OM) from the calves' faeces was calculated according to LUKAS et al. (2005). The average consumption of concentrates in the first 8 weeks after the start of supplementary feeding was 787 g per animal per day. The faeces of the calves showed that in the 3 samples (pools) before supplementary feeding, only low starch contents of a maximum of 6 g per kg DM were determined. With the start of supplementary feeding, there was a significant increase in the starch content in the faeces to over 18 mg per kg DM of faeces. After the peak on day 1 after feeding, the starch content in the feces ranged between 9-11 mg per kg DM. Overall, the use of the supplementary feed can also be assessed as positive from the point of view of the key figures in the feces, especially since the systematic preparation of the calves from suckler cow husbandry for the feeding conditions in the subsequent fattening period seems sensible and also worthwhile.

Investigation of immune parameters, health indicators and growth of German crossbred lambs exposed to natural gastrointestinal nematode infection

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Gastrointestinal nematode (GIN) infections are of major concern in global sheep farming as they cause massive production losses, treatment costs and impaired animal welfare. The increasing resistance of GIN to anthelminthics has even exacerbated this issue. Since grazing animals are inevitably exposed to GIN, pasture management, selective treatment strategies and breeding for an improved immunity of sheep against GIN are of increasing relevance. Knowledge about the complex infection mechanisms on pasture is essential as well as the identification of systematic effects that may provide guidance for appropriate modelling in quantitative genetic analyses of traits related to natural GIN infections. The aim of the study was to identify significant influences on such traits in crossbred lambs (German Merino x different meat sire breeds). A dataset of 118 lambs originating from a field trial conducted in 2022 at one experimental farm in Southwestern Germany was used. Lambs were raised indoors and subsequently managed in two grazing systems (intensive/extensive) on pasture. After six weeks of natural exposure to GIN, blood and faecal samples were collected at an average age of 11 weeks. Diarrhoea indicators were visually scored. All animals were frequently weighed during the experiment to derive average daily gain. The further phenotypes were diarrhoea indicators (Dag Score, faecal consistency score), haematological traits as well as immunological blood cell counts including eosinophils which play a substantial role in the immune response towards GIN. A mixed linear model was then applied within asreml-R. A random animal effect was included and variance-covariance structures were modelled by a genomic relationship matrix based on 50K genotypes. Systematic environmental effects were included as fixed effects and tested for significance ($p < 0.05$) in a backward elimination approach and using Wald statistics. The tested effects thereby referred to the animal (e.g. sex), the infection (faecal egg counts of the most abundant nematodes, the lambs' co-infection status of cestodes and coccidia), the general pasture system and cortisol concentration as a marker for a putative acute stress response to handling which may influence immune cell counts. In general, the infection related effects were identified as most impactful with all traits, except Dag Score, being significantly influenced by at least one of them. For example, a significant influence of Nematodirus spp. was observed on blood eosinophils and on body weight which was additionally significantly influenced by Trichostrongylidae whereas the genus Strongyloides spp. showed a significant effect on the haematological traits. The effect of the pasture system was not significant for any of the blood based traits but for average daily gain and faecal consistency score. The animal effect was significant for all traits which points to a genetic component. These results underline the complexity of natural mixed GIN infections and how phenotypic trait expression is shaped. They emphasise the importance to further investigate such traits under natural infection conditions on pasture in purebred populations with larger sample size. This is planned in a current follow-up study with several sampling timepoints to further infer the dynamics of infection and immune response.

Transforming Dairy Systems in APHCA Countries: Productivity, Health and Climate-Smart Innovation

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The member countries of the Animal Production and Health Commission for Asia and the Pacific (APHCA)—Australia, Bangladesh, Bhutan, South Korea, India, Indonesia, Iran, Lao PDR, Malaysia, Mongolia, Myanmar, Nepal, Pakistan, Papua New Guinea, Philippines, Samoa, Sri Lanka and Thailand—encompass diverse dairy systems contributing to growing global milk demand. Asia accounts for over 40% of global milk production, with continued growth driven by population increase, urbanization and dietary change. Demand is rising particularly for fresh and processed dairy products in urban areas. This study aims to assess productivity, animal health constraints and climate-related risks across representative systems and to identify priority investment pathways. Production systems range from pastoral (Mongolia, Pakistan, Iran) to smallholder mixed systems (India, Bangladesh, Nepal, Bhutan, Sri Lanka, Myanmar, Lao PDR), peri-urban systems (Indonesia, Philippines, Malaysia), and high-input commercial systems (South Korea, Australia). The analysis focuses primarily on smallholder and peri-urban systems, where most production occurs and constraints are often more pronounced, using commercial systems as reference points. The assessment draws on FAO databases, national sources and field observations from APHCA member countries, applying standardized indicators of productivity, reproduction and health, alongside emission-related metrics. Available evidence, although variable in quality and coverage, suggests that milk yields in smallholder and peri-urban systems are generally lower than in commercial systems, with substantial variation across countries and contexts. Productivity constraints are associated with feed limitations, reproductive inefficiencies and disease burdens, including mastitis and parasitism. Climate variability, input costs and infrastructure gaps further affect system performance. Data systems remain fragmented in many countries, with limited implementation of herd recording and inconsistent alignment with international standards. Animal health service access is uneven, and milk is often marketed informally or used for household consumption, limiting traceability and quality control. These findings should be interpreted cautiously given differences in data availability and system heterogeneity. Potential interventions include genetic improvement, feeding strategies, reproductive management, animal health programs, and improved housing and cooling. Strengthening data systems, extension services, milk quality assurance and value chains may support gradual improvements. Overall, the analysis suggests that integrated approaches addressing productivity, animal health and management may contribute to improved system performance, although outcomes depend on local conditions and adoption. The findings are indicative and highlight the need for improved data and longitudinal evidence to support investment planning.

Scaling up strategic investments in the dairy sector using the LSIPT WebApp: Evidence from a national genetic breeding centre in Bhutan

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It is often challenging to use science to inform policy decisions because research outputs often arrive too late in the policy cycle and require substantial analysis before they can be translated into clear policy actions – actions that may still fail to secure stakeholder buy-in. One solution is to rely on simpler tools that facilitate evidence-based, participatory dialogue among stakeholders, providing rapid though robust results, including visualizations for decision-makers. For the livestock sector, one such tool is the Livestock Sector Investment and Policy Toolkit (LSIPT) WebApp. This paper presents its application in Bhutan and shows how it generated rapid and clear evidence to support investment in a national breeding program for the dairy sector. The LSIPT has been widely used by the World Bank, FAO, CIRAD and ILRI to provide policy and invest advice to the livestock sector. Using the model, however, requires skills which often made it difficult local ownership. FAO and CIRAD have thus translated LSIPT into a user-friendly WebApp that facilitates stakeholder engagement. The LSIPT WebApp helps stakeholders build consensus on priority livestock investments: it describes current production systems and their likely evolution under a business-as-usual scenario, quantifies the expected financial and economic impacts of proposed investments, and provides summary indicators – such as Net Present Value, profit margins, and poverty or food-security outcomes – to support evidence-based decision-making. In Bhutan, LSIPT first assisted stakeholders in portraying the business-as-usual scenario of the current dairy production system – e.g. herd size and composition, quantity and price of inputs and outputs – and its expected evolution over a 20-year period. It was then used to simulate the impact of two investments at farm level: (i) the installation of a herringbone milking machine, and (ii) importation of purebred Jersey heifers to improve genetic potential. Results indicate that installation of a milking machine increases milk yield and reduces production costs, with a profit margin of 21% and reaching breakeven within five years. The introduction of improved genetics further increases production, assuming appropriate herd offtake management and adaptation of exotic animals to local conditions. Both investments are anticipated to generate positive returns and are bankable in the Bhutan context, suggesting market-based policies could trigger a transformation of the dairy sector. The participatory and user-friendly nature of the LSIPT WebApp enabled stakeholders – including government officials, technical experts, and financial institutions – to jointly explore the impact of alternative investments, strengthening shared understanding of the dairy production systems and facilitating a discussion around the impact of the proposed interventions on the technical and financial parameters more than around the description of the current production systems. This process enhances ownership of results and effectively informs strategic planning. The tool has been used in 17 countries and supports prioritization of livestock policies, efficient resource allocation, and development of investment plans engaging both public and private sectors. Initially developed collaboratively by the World Bank, CIRAD, and FAO, the transition from an Excel-based research tool to a web-based platform translated in several languages has expanded accessibility to a broader community of animal scientists and policymakers.

Building Inclusive and Sustainable Dairy Production: Global Insights on Community-Led Transformation Models from Heifer International

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Smallholder dairy systems across low- and middle-income countries continue to face persistent constraints in genetics, feeding, climate resilience, and market access. Heifer International's community-led dairy models show that integrated genetic improvement, climate-smart production systems, stronger cooperatives, and market-aligned incentives can deliver rapid productivity gains and build more resilient dairy economies. In Nepal, the Milky Way Program— the country's national initiative to transform its dairy sector—has established a smallholder-managed Model Dairy Village (MDV) as a center of excellence and learning. More than 500 households have adopted advanced dairy technologies and improved management practices through the MDV. A cohort of one hundred Holstein Friesian (HF) heifers imported from South Korea is now producing an average of 5,742 liters per 305-day lactation—nearly 80 percent higher than existing HF crossbreeds in Nepal. Top-performing animals are reaching up to 9,000 liters per lactation, comparable to HF performance levels in Korea. Technologies and best practices proven in the MDV are being disseminated to more than 40,000 dairy-farming households nationwide, in partnership with the Central Dairy Farmers Federation and 120 milk producer cooperatives. Cooperative-led Business Development Services have raised daily milk collection to 1,800 liters, with a plan to reach 10,000 liters per day to support a viable domestic dairy industry and reduce approximately USD 17 million in dairy import costs. The program has also established Nepal's first Holstein Friesian nucleus herd within the MDV to generate high-genetic-merit dairy animals. This herd ensures a steady supply of quality breeding bulls for semen production at the Government's National Livestock Breeding Office (NLBO). To date, eleven high-potential male calves from the MDV have been procured by the NLBO to support national genetic improvement. In addition, the national breeding system has been strengthened through the production and utilization of more than 275,000 frozen semen doses from eight imported HF bulls. Over time, these measures will raise the genetic quality of Nepal's dairy herd, increase milk productivity, and improve farm incomes across the country. Across Africa, Heifer supports inclusive dairy development by strengthening farmer cooperatives, upgrading extension and animal-health systems, and expanding climate-smart forage production. In Rwanda, dairy productivity indicators have improved significantly, with average daily milk yield increasing from 3.5 to 5.9 liters per cow (≈+69%) and the share of milk sold rising to 70% of total output. These gains are supported by 50 new milk collection points and improved market connectivity within one of Heifer's projects. In Honduras, Heifer-supported sustainable livestock practices have increased dairy productivity by about 25%, improved land-use efficiency by 14–32%, enhanced water-use efficiency by 21–48%, and reduced greenhouse-gas emission intensity by 14–16%. These improvements are particularly important in Olancho, where 82% of producers report climate-related impacts such as drought and heat stress. More than 57% of producers are already implementing at least basic sustainable practice packages, and wider adoption could deliver an additional ~30% productivity gain and a 15–17% reduction in emissions intensity. Together, these regionally diverse cases demonstrate how Heifer International's community-centered, integrated dairy system interventions can produce scalable improvements in productivity, environmental performance, and household income and resiliency across varied production systems.

Enhancing dairy productivity in low- and middle-income countries through synergistic approaches

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Low dairy productivity in many low- and middle-income countries (LMICs) is largely driven by limited availability and poor quality of feed, strong seasonal fluctuations in forage supply, low animal productivity, and increasing climate variability. Improving feed systems is therefore central to enhance dairy productivity, strengthening resilience, and reducing greenhouse gas emission intensity. The ILRI (International Livestock Research Institute) aims to impact the livelihoods of millions of smallholder farmers in LMICs by enhancing dairy productivity through climate-resilient approaches that improve feed availability, quality, and utilization, thereby generating synergistic gains in animal performance and health. Our key intervention areas include: (1) the identification and promotion of productive, nutritious, climate-resilient, low-methane forage species through advanced laboratory and field evaluations, together with molecular genetics and genomic tools to capture and utilize available genetic diversity, including materials conserved in genebank collections; (2) Use of alternative feeds, including application of strategic supplementation; (3) crop-livestock integration practices that increase biomass production and improve feed nutritive value of crop residues, including the strategic supplementation of residues with legumes or concentrates for a more balanced nutrient supply; (4) improved feed conservation techniques to reduce seasonal feed shortages; and (5) greater utilization of locally available feed resources to enhance resilience to climate variability. These interventions are integrated with dairy genetics and animal health technologies to generate synergistic improvements in productivity, health, and feed efficiency. Integrated "best-bet" technology packages are being developed and promoted, and subsequently scaled through multiple delivery pathways, including digital platforms and artificial intelligence-supported decision tools. The approach also integrates economic and policy dimensions, including livestock master plans, investment opportunities, and inclusive business models that support scaling of improved feeding and dairy practices. Particular attention is given to gender- and youth-responsive approaches to strengthen participation in emerging milk, feed and forage value chains. Together, these integrated strategies provide a pathway for sustainable intensification of dairy systems in LMICs while improving livelihoods and environmental outcomes. In this presentation, we aimed to share our experiences and strategies in addressing key challenges and constraints through coordinated research and development interventions. Presenting this synthesis at the annual meeting of the European Federation of Animal Science (EAAP) provides an opportunity to share lessons from LMICs and contribute to broader scientific discussions on sustainable dairy production. Keywords: Sub-Saharan Africa, sustainable dairy systems, improved feed system, unified approach, climate resilience.

Delivering Genetic Gains for Productivity, Climate Resilience, and Low Emissions in African Dairy Systems through ILRI-Led Genomics-Based Breeding

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African dairy production systems face the intertwined challenges of low productivity and high climate vulnerability resulting in low profits for smallholder farmers. Drawing on flagship initiatives including the Africa-Asia Dairy Genetic Gains program and Enviro-Cow project, this paper summarizes ILRI's genomics-enabled and digital approaches to genetic gains while delivering low-emission, resilient, disease resistant, and productive dairy cattle for dairy systems across Africa. The phenotypic data collected includes milk yield, body weight, milk quality traits, somatic cell score, reproductive performance, and CH_4 recorded on smallholder farms across Kenya, Ethiopia, Tanzania, and Uganda. This is collected using digital tools developed using the Open data kit (ODK) and a customized mobile app developed for smallholder farming systems. Animals are genotyped and genomic Estimated Breeding Values (GEBVs) are computed using a single-step Genomic Best Linear Unbiased Prediction (ssGBLUP) method. Top ranked bulls based on their GEBVs are selected and promoted for use through artificial insemination. Key results from the enviro-cow project to date include: (i) preliminary heritability estimates for enteric CH_4 of $h^2 = 0.19 \pm 0.04$ (genomic HBLUP, year-season averaged model) based on 945 crossbred dairy cows with 15,723 Laser Methane Detector records and 459 genotyped animals (90,407 SNPs) across Ethiopia and Tanzania— estimates comparable to global dairy populations ($h^2 = 0.20–0.30$). Building on these findings, the ongoing Global Methane Genetics (GMG) Initiative at ILRI aims to advance innovations in breeding climate-smart dairy cattle across Kenya, Ethiopia, South Africa, Burkina Faso, and Benin, phenotyping approximately 4,255 animals and collecting 1,000 rumen microbiome samples with the goal of demonstrating that Africa can lead in reducing livestock methane emissions through genetic improvement. Evidence generated by ILRI and its partners across diverse dairy production systems in East Africa shows that data-driven breeding programs can substantially increase rates of genetic gain while enhancing climate resilience and livelihood outcomes. We further highlight the critical role of institutional innovation, inclusive breeding strategies, public–private partnerships, and national breeding program development in enabling effective implementation. Future activities will focus on expanding genomic evaluations to national and regional systems and embedding climate-smart and low-emission selection indices into routine national genetic evaluations. These efforts are expected to measurably improve smallholder productivity, strengthen climate resilience, and contribute to regional and global dairy sustainability targets.
Keywords: Genomic selection, methane emissions, rumen microbiome, digital phenotyping, smallholder systems

Investing in dairy for inclusive rural transformation: priorities and challenges from different regions and systems

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Dairy production is a key pathway for inclusive rural development, contributing to income generation, nutrition, and employment for millions of rural households worldwide. As demand for milk and dairy products continues to grow, particularly in Africa and Asia, investments in sustainable dairy systems are becoming increasingly important for strengthening food systems and improving rural livelihoods. This presentation builds on 5 decades of dairy development in the IFAD as part of its mandate to reduce rural poverty and enhance food security by enabling smallholders, pastoralists and rural entrepreneurs to build resilient and profitable agricultural livelihoods. For example, in East Africa, where dairy production is largely dominated by smallholder farmers, investments focus on strengthening productivity and value chains. This includes promoting improved dairy breeds, better feed and forage systems, access to animal health services and stronger farmer cooperatives. These interventions help smallholders increase milk yields, improve milk quality and access reliable markets, while also supporting inclusive rural livelihoods, particularly for women and youth. In Central Asia, dairy production is based on pastoral and agro-pastoral systems that rely on seasonal grazing and extensive livestock management. Development programmes emphasize sustainable rangeland management, improved "last-mile" and remote veterinary and breeding services, and better access to diversified markets for livestock products. Strengthening pastoral value chains and community-based resource management helps improve livestock productivity while maintaining the resilience of pastoral livelihoods and ecosystems. In Eastern Europe and the Caucasus, dairy sectors are characterized by emerging medium to large scale farms and evolving market structures. Increasing farm productivity while strengthening milk quality and safety standards, and supporting modern processing and market linkages, is essential. These investments help producers meet growing consumer demand while enhancing competitiveness and sustainability in regional dairy industries. Environmental sustainability should be a systematic requirement in dairy investments. Livestock systems are closely linked to land, water and biodiversity resources, and well-managed dairy production can contribute to sustainable landscape management. Many programmes promote integrated crop–livestock systems, improved manure management and sustainable pasture practices that enhance soil fertility, reduce environmental degradation and improve resilience to climate shocks. This can be facilitated by leveraging supplementary funds such as the Green Climate Fund, the global Environment Facility or the Adaptation Fund to co-finance development projects, provide technical assistance and support environmental outcomes. One health approaches are also key to support pandemic prevention, preparedness, and response (PPR) initiatives, especially in rural communities. This should not only focus on animal health and disease surveillance but also include the health of ecosystems (e.g. pasture restoration) and the management of livestock-wildlife interactions. Dairy development also offers important opportunities for social inclusion. Women often play a central role in dairy production, milk processing and marketing, making the sector particularly relevant for women's economic empowerment. Dairy development programmes frequently include targeted support for women and youth, including access to training, financial services and leadership roles within producer organizations.

Innovations in temperate grazing dairy systems in Australia driven by precision management of pasture and livestock

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Australian dairy production systems are predominantly pasture-based, with farm performance and productivity largely determined by the efficient utilisation of pasture and livestock, in contrast to confinement systems reliant on capital-intensive infrastructure (e.g., housing and TMR infrastructure). A major constraint to land-use efficiency is difficulty of consistently implementing optimal grazing management. Pasture utilisation is maximised when grazing is aligned with plant physiological stage, typically around the 2 to 3-leaf stage in temperate systems, and when post-grazing residuals are maintained at levels supporting regrowth while minimising wastage. In practice, labour and operational constraints often limit the ability to achieve this level of precision. Virtual fencing technologies (e.g., Halter) provide a promising tool by enabling automated and flexible control of animal movement. This allows more consistent timing of grazing events and improved control of post-grazing residuals, reducing both under- and over-grazing. Thus, optimised grazing timing and residual management can increase pasture growth and grazed harvest, with anecdotal evidence suggesting increases in home-grown forage production of approx. 10–20%, where the technology is effectively applied. Importantly, these benefits are achieved with reduced labour input, increasing the likelihood that optimal grazing strategies are consistently implemented while contributing to improved labour efficiency and farmer wellbeing. Complementing improvements in land utilisation, wearable technologies target the livestock asset by enabling more precise, individualised animal management. Advanced identification and feeding systems allow delivery of customised feed amounts and formulations to individual cows within a herd during milking sessions, for example by increasing feed allocation to postpartum cows and reducing inputs for cows in later lactation. At the animal level, rumination monitoring provides reliable, real-time indicators of rumen function, enabling earlier detection of nutritional imbalances than traditional approaches used to identify conditions such as subacute ruminal acidosis, which are typically based on production responses such as milk yield and composition, and visual assessment such as faecal characteristics. This is particularly valuable in grazing systems where highly digestible, low-structural fibre spring pastures, and large concentrate feeding, may increase the risk of ruminal dysfunction, and where decisions about effective fibre provision are often uncertain despite adequate total grazed feed availability. Overall, competitiveness in grazing dairy systems is fundamentally driven by efficient pasture utilisation and animal productivity. These technologies can enhance control over these key assets by improving pasture management and enabling more responsive, animal-level interventions, thereby supporting increased productivity per hectare and per cow while maintaining the low-capital structure underpinning the efficiency and resilience of Australian grazing enterprises.

Production Efficiency Trends in Thai Dairy Farms through Strategic Benchmarking

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The Thai dairy industry between 2023 and 2025, comprising 15,300 farming households and a national milking herd of 247,900 cows, is a vital agricultural sector producing approximately 1.05 million tons of raw milk annually. The efficiency of Thailand's dairy production, however, is under considerable pressure from high input costs and climate-related impacts, requiring effective transformation strategies. While literature suggests that benchmarking is a powerful tool for performance improvement, the practice remains relatively new to the Thai dairy industry. Consequently, the Thailand–Australia partnership project on dairy farm benchmarking has been established to facilitate precision farm management, thereby reducing the risk of financial loss of dairy farm business. This study aimed to monitor progress in the production efficiency of participating dairy farms. A framework of twenty benchmarks was developed based on preliminary findings, where Principal Component Analysis was applied to check multicollinearity and identify primary drivers of efficiency. Data were categorized into herd structure, production performance, and feeding inputs. Milk income over feed cost (MIOFC) was selected as the key comparative variable and was validated using the Pearson correlation coefficient to demonstrate its relationship with other efficiency indicators. Farms in the top quartile of MIOFC were classified as frontier farms, and the remaining 19 variables were analyzed in relation to this classification. Linear mixed models, controlling for variability at farm level, were utilized to identify significant performance differences across years. Results revealed six benchmarks that differed significantly across years ($p < 0.01$), regardless of farm system variability, which were total herd size, milk yield per cow per day, milk fat, feed cost, milk price, and milk revenue. Average herd size increased significantly from 62 in 2023 to 81 in 2025. Furthermore, a 10% higher ratio of replacement heifers to cows observed in 2025 may indicate a growing emphasis on genetic improvement. Although overall production efficiency improved, high feed costs which accounted for 64.8% of milk revenue remained a primary concern. Analysis by milk yield quartile showed that the average yield in the lowest quartile was 11.7 liters per cow per day. Yields below this level of production could be associated with a high risk of financial loss once other production costs were considered. The observed transition toward larger herd sizes and improved MIOFC, driven by gains in daily milk yield and components, indicated a structural intensification against rising input costs. However, the high proportion of feed costs relative to revenue emphasized the continued financial vulnerability of small-holder farm systems. The identification of this yield level provides a critical benchmark to sustain farm business, as farms producing below this level are unlikely to achieve a positive net profit margin. In conclusion, the monitored benchmarks provide a tool to track production efficiency and implement precision management strategies which could lessen the current poor terms of trade in the Thai dairy sector.

The natural additive Fator P included in diets of increases affinities of antibodies specific for salivary proteins of Rhipicephalus microplus ticks in beef and dairy cattle

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Infestations by R. microplus are responsible for reducing meat and milk productivity in cattle farming. Parasitism by ticks is mediated by a large repertoire of salivary proteins that they secrete into the blood pool they make in their hosts' skin. Modern management practices combined with precision nutrition have been an important strategy to curb the spread of tick populations by strengthening animal health, increased resistance against ectoparasites and reduced herd infestation in a sustainable manner. In this case, the inclusion of natural additives in the diet can enhance the maturation of the immune response, to increase the functional affinity of antibodies against R. microplus antigens and to modulate the host's immune system. Fator P is a natural zootechnical feed additive that has been shown to improve several production features in beef and dairy cattle. Due to its composition and to the mechanisms by which vertebrate hosts generate high affinity specific antibodies, we hypothesised that Fator P improves the affinities of saliva-specific antibodies in bovines of a genetically tick-susceptible breed. In order to test this hypothesis in part, we measured levels and affinities of antibodies specific for select recombinant tick salivary antigens in blood from bovines that had been treated or not with Fator P and infested artificially with unfed larvae of R. microplus. Purebred, castrated Holstein steers, aged 18 to 20 months were randomized into two groups (N= 8/group) based on weight, body condition score, paternity and innate tick loads. The study was blinded and both groups were confined in individual pens in a feed lot with an empty pen separating each animal; the test group received Fator P (dosed at 6,0 gr / animal / day) and both groups received 2,2% of body on a dry matter (DM) in diet. Blood samples for serum were collected on days 28, 56, 63, 70, 87, and 119 after the start of treatment with the additive. Avidity was measured using 6, 7, 8 or 9 M urea washes in a conventional ELISA that employed recombinant antigens cloned from genes encoding select secreted proteins from salivary glands of R. microplus. The statistical method used was the Mann-Whitney test, with p-values <0.05. Sera collected on the first day of treatments contained saliva-specific antibodies that presented a similar percentage of avidity amongst both groups ($p=0.9214$). After the 28th day of treatment, the percentage of antibody avidity increased significantly, from 0.09% to 0.27% ($p<0.0001$) for the group treated with Fator P and from 0.10% to 0.11% for the control group. After that, the results remained constant until day 119, varying from 0.30% to 0.55% ($p<0.0001$) and from 0.10% to 0.11% for the treated and control group animals, respectively. Fator P promoted a significant increase in the avidity of saliva-specific antibodies in treated cattle. This process depends on affinity maturation of antibodies in germinal centres (GCs) of lymph nodes, and Fator P may improve it because B lymphocytes do not rely on aerobic glycolysis but actively oxidize fatty acids when hyperproliferating in GCs. We predict that natural additive Fator P can action on precision nutrition on targets metabolic processes that improve antibodies responses, including to tick saliva, explaining the reduction of tick loads that is also observed in bovines that receive this additive.

Mitigation of enteric methane emissions by dietary essential oil supplementation in tropical feedlot systems

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Enteric methane (CH_4) emissions from beef cattle represent a major contributor to the carbon footprint of livestock systems and a loss of dietary energy. Developing effective mitigation strategies for tropical production systems is therefore essential to improve the environmental sustainability of beef production. Essential oil (EO) compounds have emerged as promising rumen modulators capable of altering microbial fermentation pathways and reducing methanogenesis without compromising animal performance. This study evaluated the effect of a plant-derived feed additive containing carvacrol and capsaicin (ValoPro-E) on productive performance and enteric CH_4 emissions of feedlot-finished Nellore bulls under tropical conditions. Forty-eight non-castrated Nellore bulls (initial body weight = 333.6 ± 4.1 kg) were assigned to two treatments (n = 24): control (basal diet + mineral premix) or EO supplementation (basal diet + mineral premix + 3 g/animal/day of additive). Diets were formulated according to NASEM (2016) with a forage-to-concentrate ratio of 12:88. Animals were housed into collective pens equipped with automated bunks and scales (Intergado®), allowing individual monitoring of feed intake and body weight. Enteric CH_4 emissions were measured in a subset of animals (n = 12 per treatment) using the sulfur hexafluoride (SF_6) tracer technique with continuous 24-h sampling for five consecutive days. Data were analyzed using mixed models with treatment as fixed effect and initial body weight as covariate ($P < 0.05$). Supplementation with EO did not affect dry matter intake, final body weight, average daily gain, feed efficiency, hot carcass weight or dressing percentage ($P > 0.05$). Generalized additive models indicated that daily BW trajectories differed between treatments ($P = 0.003$), with a plateau in weight gain during the final days observed in treatment control but not in EO-treated animals. However, EO supplementation reduced absolute CH_4 emissions compared with control group (174.6 vs. 193.1 g CH_4/day; $P = 0.002$), representing a 9.5% reduction in enteric CH_4 production. In addition, CH_4 intensity was reduced relative to carcass weight ($P = 0.002$), final body weight ($P < 0.001$), and CO_2-equivalent emissions per kilogram of meat produced ($P = 0.02$) for EO-supplemented animals. Methane yield (CH_4/DMI) was not affected. These results demonstrate that EO supplementation can reduce enteric CH_4 emissions while maintaining productive performance in tropical feedlot systems. This strategy represents a practical nutritional approach to support climate-efficient beef production in tropical regions, contributing to the mitigation of greenhouse gas emissions in the global livestock sector. This study was supported by FAPESP (grants 2017/50339-5, 2021/11922-2) and CNPq.

Effect of 3-NOP and dietary NDF concentration on methane emission and ruminal synthesis of vitamin B12 in dairy cows

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Dietary inclusion of 3-NOP, through its inhibition of methanogenic archaea activity, combined with a low dietary concentration of neutral detergent fiber (NDF), which is positively associated with ruminal vitamin B12 synthesis, may increase the risk of impaired ruminal synthesis of vitamin B12. The present study aimed to evaluate the effect of dietary NDF concentration in combination with 3-NOP on methane emissions, and ruminal synthesis of vitamin B12 in dairy cows. Four multiparous Danish Holstein cows were fitted with a rumen cannula and a T-shaped cannula in the duodenum. The study design was a 4×4 Latin square with 4 treatments over 4 periods of 21 days. Each period consisted of 14 days of adaptation, 3 days of digesta collection, and 4 days of gas exchange measurements in respiration chambers. The basal diets (g/kg DM) consisted of 505 grass silage, 136 rapeseed meal, and 10 mineral and vitamin supplements combined with either 350 barley (low NDF diet; LoNDF) or 350 dried sugar beet pulp (high NDF diet; HiNDF) and were supplemented with either 0 or 60 mg 3-NOP/kg DM (Bovaer). Therefore, cows were fed four diets as follows: LoNDF, LoNDF_3-NOP, HiNDF, and HiNDF_3-NOP. Dietary NDF concentrations (g/kg DM) were 312 ± 21 and 371 ± 12 (mean ± SD) in LoNDF and HiNDF diets, respectively. Eight duodenal samples were collected at 8–10 h intervals, pooled, and analyzed for vitamin B12. Dry matter intake (DMI; kg/d) affected by dietary NDF concentration ($P < 0.01$) and was higher in the low NDF diets (22.3) compared to the high NDF diets (20.4). Methane production (g/d) was 23% lower ($P < 0.01$) in the diets supplemented with 3-NOP (334) compared to the non-3-NOP supplemented diets (432). Methane yield (g/kg DMI) was 22% lower ($P < 0.01$) in the diets supplemented with 3-NOP (15.8) compared to the non-3-NOP supplemented diets (20.2). Hydrogen (H2; g/d) production was affected by 3-NOP ($P < 0.001$), and it was higher in the diets supplemented with 3-NOP (9.9) compared to the non-3-NOP supplemented diets (0.6). Duodenal concentrations of all corrinoids (i.e. B12-like molecules = cobalamin + analogues; nmol/kg DM) were 2742, 2527, 3302, and 3222 for LoNDF, LoNDF_3-NOP, HiNDF, and HiNDF_3-NOP, respectively and were affected by dietary NDF concentration ($P < 0.01$). Duodenal concentrations of the active vitamin B12 (i.e. cobalamin) were affected by dietary NDF concentration ($P < 0.01$), with higher concentrations in the high NDF diets (839 nmol/kg DM equal to 1.14 mg/kg DM) compared with the low NDF diets (592 nmol/kg DM equal to 0.80 mg/kg DM). There was no interaction between dietary NDF concentration and 3-NOP for the aforementioned variables. In conclusion, DMI increased when dietary NDF concentration was reduced. Methane production and methane yield were lower in the diet supplemented with 3-NOP. The findings indicate that dietary NDF concentration is a major driver of rumen vitamin B12 synthesis, as increasing dietary NDF increased duodenal vitamin B12 concentration without any concurrent adverse effect of 3-NOP.

Effects of 3-nitrooxypropanol (3-NOP) supplementation on enteric methane emissions and milk production in dairy cows fed canola meal or soybean meal

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The objective of this study was to evaluate the effects of including 3-nitrooxypropanol (3-NOP; Bovaer 10®; dsm-firmenich, Switzerland) in dairy cow diets based either on soybean meal (SBM) or canola meal (CM) on enteric CH4 emissions (production, yield, and intensity), and milk performance (production and composition). Twelve multiparous Holstein cows were used in a replicated 4 × 4 Latin square design (35-d periods; 14-d adaptation) with a 2 × 2 factorial arrangement of treatments. Cows were fed ad libitum (5% refusals, as-fed basis) a TMR containing either SBM or CM as the main protein source, without or with 3-NOP (60 mg/kg of diet DM). The diets were isonitrogenous, and CM (23% of diet DM) replaced SBM and soybean hulls. Milk production and milk composition were measured for 7 d, and CH4 emissions were measured for 4 d using respiratory chambers. Data were analyzed using the MIXED procedure of SAS with fixed effects of meal source (CM vs. SBM), 3-NOP (with vs. without) and their interaction; square and cow within square were considered random effects. Significance was declared at $P \leq 0.05$. No meal source × 3-NOP interaction was detected for any of the response variables. Dry matter intake (DMI) was unaffected by the treatments, averaging 29.9 kg/d. Milk yield tended ($P = 0.09$) to be greater in cows fed CM (+1.1 kg/d), but was unaffected by 3-NOP. Yields of energy-corrected milk (ECM) and milk components (fat, protein, and lactose) were unaffected by the meal source or 3-NOP. However, cows fed CM exhibited lower MUN concentration than cows fed SBM (12.1 vs. 13.3 mg/dL; $P < 0.01$). Replacing SBM with CM reduced enteric CH4 production (g/d), yield (g/kg DMI) and intensity (g/kg ECM) by 12% on average. Supplementation with 3-NOP reduced CH4 production, yield, and intensity by 28% compared with unsupplemented cows. In conclusion, replacing SBM with CM tended to increase milk yield (+2.5%) and significantly reduced enteric CH4 emissions (-12%), while also lowering MUN concentration, suggesting improved nitrogen utilization. Regardless of meal source, dietary supplementation with 3-NOP decreased CH4 emissions by 28% without affecting intake or milk production. The combined strategy of feeding CM and 3-NOP reduced enteric CH4 production, yield, and intensity by an average of 36% compared with feeding SBM without 3-NOP.

Effects of 3-nitrooxypropanol, nitrate, and rumen-protected fat, alone and in combination, on gaseous emissions and performance of lactating dairy cows

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3-nitrooxypropanol, nitrate and rumen-protected fat have shown anti-methanogenic properties in ruminants, each following a different metabolic mode of action. However, their combined effects are less clear. Hence, our aim was to evaluate the addition of 3-nitrooxypropanol, nitrate and rumen-protected fat, either alone or in combination, to the diet of lactating dairy cows. We hypothesized that the CH4 mitigating effects of the individual interventions would be additive when combined. The experiment followed a randomised complete block design and included a 2-wk covariate period for baseline measurements followed by a 12-wk treatment period. Sixty-four Holstein-Friesian dairy cows (130 ± 27 DIM and 33 ± 5.6 kg/d fat- and protein-corrected milk (FPCM) at the start of trial; mean ± SD) were blocked by parity, DIM, and FPCM yield, and randomly allocated to 1 of 8 treatments: a control diet (CON; 39% grass silage, 27% maize silage and 34% concentrates on DM basis), the individual addition of Bovaer® (3-nitrooxypropanol, 3NOP), SilvAir® (nitrate, NIT) or Mega-Max 2.0® (rumen-protected fatty acids, FAT), the combination of 2 of these compounds (3NOP+NIT, 3NOP+FAT, or NIT+FAT), and a treatment combining all 3 compounds (3NOP+NIT+FAT). The realized doses were 68.4 mg 3NOP/kg DM, 14.1 g NIT/kg DM and 23.8 g FAT/kg DM. Measurements of CH4, H2, and CO2 were performed using 3 GreenFeed units. Data were analysed using repeated-measures mixed models in R, including treatment, time, and their interaction as fixed effects, with block as a random effect and cow nested within block as a repeated factor over time. Preliminary results show that total DMI was lower ($P < 0.01$) for both 3NOP+NIT (20.7 kg/d) and 3NOP+NIT+FAT (20.8 kg/d) compared to CON (22.9 kg/d). Methane yield (g/kg DMI) was not significantly affected by FAT, but was reduced ($P < 0.01$) by 20.1% for 3NOP and 20.7% for NIT compared to CON (20.8 g CH4/kg DMI). Dual combinations of 3NOP, NIT, and FAT did not result in significantly larger reductions of CH4 yield compared to 3NOP and NIT alone. Combining all 3 interventions achieved the largest inhibition of CH4 yield (-30.4%) differing from all other treatments ($P < 0.01$). Similar responses were observed for H2 yield, which did not differ between FAT and CON (0.05 g/kg DMI for both), but increased ($P < 0.01$) with NIT (0.12 g/kg DMI) and 3NOP (0.21 g/kg DMI). Dual combinations containing 3NOP or NIT did not increase H2 yield beyond that of 3NOP or NIT alone, respectively. Also, combining all 3 interventions resulted in a similar H2 yield level as that of 3NOP alone. None of the treatments differed from CON for CO2 yield, but the CO2 yield of FAT (631 g/kg DMI) was lower than both 3NOP+FAT (677 g/kg DMI) and 3NOP+NIT (675 g/kg DMI; $P = 0.05$). The FPCM yield was higher for FAT (36.2 kg/d) and lower for 3NOP+NIT (31.4 kg/d) compared to CON (34.8 kg/d; $P < 0.01$). Depending on the combination of 3NOP, NIT and FAT, reductions in CH4 emissions did not always appear additive and were sometimes accompanied by numerical decreases in DMI or FPCM (e.g., 3NOP+NIT). Conversely, some combinations showed trends towards greater CH4 inhibition with less impact on DMI or FPCM (e.g., when combining FAT with 3NOP or NIT) or smaller increases in H2 yield (e.g., NIT+3NOP compared with 3NOP). Understanding the mode of action of each intervention and its effects on rumen function and post-absorptive metabolism is key to adequately recommending combinations of 3-nitrooxypropanol, nitrate and rumen-protected fat.

Exploring the effects of 3-nitrooxypropanol in diets varying in crude protein and starch content on methane and nitrogenous greenhouse gases emissions from dairy cows

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This study aimed to investigate additive effects of 3-nitrooxypropanol (3-NOP) in diets varying in crude protein and starch content on CH4 and nitrogenous emissions from dairy cows. In total, 64 Holstein-Friesian dairy cows were enrolled with a milk yield of 37.4 ± 7.22 kg/d (mean ± SD) at 166 ± 30 DIM. Cows were blocked in groups of 4 and within block randomly allocated to 1 of 4 mechanically ventilated barn units (mimicking regular cow housing, floor, and manure management), with cows within barn unit receiving the same treatment. The experiment lasted 10 weeks; 2 weeks covariate for baseline measurement and 2 treatment periods (both comprising 2 weeks adaptation and 2 weeks measurement). A control diet (CON) with 177 and 173 g/kg DM CP and starch, respectively, and a low protein high starch diet (LPHS) with 154 and 255 g/kg DM CP and starch, respectively were supplemented with 0 or 60 mg 3-NOP/kg DM (NOP0 and NOP60, respectively). All diets consisted of 39% grass silage, 23% corn silage, and 38% concentrates (DM basis). The differences in CP and starch content between CON and LPHS were realized by a different concentrate composition for both diets. The LPHS diet was supplemented with rumen-protected lysine (AjiPro-L) to maintain metabolizable lysine. Additional concentrate was fed in the GreenFeed system that was used to measure enteric CH4 emissions. Ventilation rates and concentrations of CH4, NH3, and N2O were measured in background and exhaust air of each barn unit. Data were analyzed on barn unit and subjected to ANOVA using the MIXED procedure in SAS. Baseline, 3-NOP, diet, and 3-NOP × diet were fixed effects, and barn unit, period and treatment sequence were random effects. No interactions between 3-NOP and diet were observed. Enteric CH4 emission and manure CH4 emission represented on average 72.5% and 27.5%, respectively, of total CH4 emissions. Enteric CH4 emissions were reduced by 22.7% ($P \leq 0.02$) with NOP60 compared to NOP0. Barn emission of NH3 and N2O were lower (-29% and -14%, respectively; $P \leq 0.03$) for LPHS compared to CON. In conclusion, enteric CH4 emissions were reduced by 3-NOP irrespectively of the dietary CP and starch content, and LPHS reduced nitrogenous emissions as result of the lower CP content.

Effects of feeding palmitic and stearic acids as triglycerides on milk production and methane emissions in dairy cows

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Dietary fat supplementation is a nutritional strategy to mitigate enteric methane (CH_4) emissions from dairy cows. However, responses depend on fatty acid type and associated effects on production. This study hypothesized that supplementation with saturated fatty acids C16:0 and C18:0 as triglycerides (TG) at increasing levels, would reduce daily enteric methane production (CH4 g/day) and increase energy-corrected milk (ECM) yield and thereby reduce methane intensity (CH4 g/ECM). It was also hypothesized that the effects were linear to supplementation level. The effects of increasing dietary concentrations of TG rich in palmitic acid (P-TG) or stearic acid (S-TG) on dry matter intake (DMI), CH_4 emissions and milk composition were studied. The experiment included 48 lactating Danish Holstein cows (24 primiparous and 24 multiparous) and was conducted at Aarhus University research facilities. Six dietary treatments were evaluated: a control diet and low (L), medium (M), and high (H) inclusion levels (1.43, 2.87, and 4.30% of dry matter) supplied as S-TG (LS, MS, HS) or P-TG (MP, HP). The diets were formulated with a 70:30 silage-to-concentrate ratio (silage: 50:50 DM grass–clover and maize silage) and offered ad libitum as a PMR. Additionally, up to 1 kg DM concentrate was provided in the GreenFeed units for enteric CH_4 measurement. Each cow received four of the six treatments, one in each period, for a total of four periods, each lasting three weeks (incomplete 6×4 Latin square). The first two weeks served as adaptation and the third week was used for sampling. Emissions of CH4 were measured using the GreenFeed system to obtain individual gas production. Daily dry matter intake (DMI) and milk yield were recorded, and in sampling weeks milk samples were analyzed for protein, lactose, and fat content. Data were analysed using a linear mixed-effects model in R (v4.4.2; lme4 package). Dietary treatment, period, days in milk (DIM), and parity were included as fixed effects, including the interactions between DIM and parity and between treatment and parity, with cow as a random effect. Model assumptions were evaluated graphically. Results are presented as estimated marginal means (emmeans), and linear and quadratic contrasts were estimated. Supplementation with P-TG increased ECM yield compared with the control (37.5 kg/d), reaching 40.0 and 40.7 kg/d for MP and HP, respectively (treatment, $p < 0.001$). Milk fat percentage and fat yield increased with P-TG supplementation (4.82% for control vs. 5.03% and 5.08% for MP and HP; treatment, $p < 0.001$). Although milk protein percentage decreased, daily protein yield increased (1.29 kg/d for control vs. 1.33 and 1.34 kg/d for MP and HP; treatment, $p < 0.001$). Feed efficiency (ECM/DMI) increased with P-TG supplementation (1.58 for control vs. 1.65 and 1.68 for MP and HP; treatment, $p < 0.001$). Consequently, CH_4 intensity decreased as a linear effect (12.1 for control vs. 11.0 and 10.7 CH_4/kg ECM for MP and HP; treatment, $p < 0.001$, linear $p < 0.001$). Total daily CH_4 emissions were not significantly affected by any fat supplementation. Supplementation with S-TG did not affect ECM or milk composition. DMI increased linearly for cows on the S-TG diet and for multiparous cows on the P-TG diet ($p < 0.001$ and $p = 0.02$ respectively). In conclusion, supplementation with P-TG increased ECM yield and feed efficiency and reduced methane intensity.

Immunonutrition in ruminants

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The Nutrition Commission proposed inviting me as a speaker. I was asked to provide this abstract and a review until September in case I get invited. In my talk/review I will link nutrition, immunology, rumen microbiome, feed efficiency and enteric methane emission in ruminants. How does nutrition influence immune and microbial function in ruminants with low/high feed efficient phenotypes and with low/high methane emitting phenotypes? How can we adjust nutrition to optimize immune and microbial function in ruminants differing in methane and feed efficiency phenotypes? The review aims to provide an overview of existing knowledge, gaps in knowledge and suggestions for moving from descriptive to mechanistic studies. This abstracts hasn't matured yet, but I highlight relevant reports. Arshad et al. (2024) reported that immune cells in high feed efficient cows (low RFI) exhibit an increased metabolic function, which might influence nutrient partitioning and utilization and serve as a source of variation in feed efficiency that warrants further investigation. In our study in press in Journal of Dairy Science (Nur Cahyo et al. 2026), high feed efficient, early-lactating cows (high ECM/DMI) showed a stronger immune response, but also a higher methane yield (high CH4/DMI). Reiche et al. (2025) reported a higher proportion of IgA-coated archaea in feces of 3-NOP fed cows compared to control cows, indicating an active role of the immune system in inhibiting rumen microbial function. Gibson et al. (2016) demonstrated a more efficient immune response in Brown Swiss compared to Holstein cows, with the former being known for a comparable lower feed efficiency and greater methane emissions. Genes but even more environmental factors such as nutrition affect feed efficiency, rumen microbiome and immunological condition. Research in methane mitigation by feeding measures focusses on feed efficiency and more recently on additive effects of multiple feed additives. Immuno-microbial-nutrition is an emerging research field, considering the relevance of immune and microbial function for the ruminant host. Scientific discipline: Ruminant Science ReferencesArshad, U., Kennedy, K.M., Cid de la Paz, M. et al. (2024) Immune cells phenotype and bioenergetic measures in CD4+ T cells differ between high and low feed efficient dairy cows. Sci Rep 14, 15993. https://doi.org/10.1038/s41598-024-66345-x Gibson AJ, Woodman S, Pennelegion C, Patterson R, Stuart E, Hosker N, Siviter P, Douglas C, Whitehouse J, Wilkinson W, Pegg S-A, Villarreal-Ramos B, Werling D (2016). Differential macrophage function in Brown Swiss and Holstein Friesian cattle, Veterinary Immunology and Immunopathology, Volume 181, 2016, Pages 15-23, ISSN 0165-2427, https://doi.org/10.1016/j.vetimm.2016.02.018. Kehrli, M. E., Neill, J. D., Burvenich, C., Goff, J. P., Lippolis, J. D., Reinhardt, T. A., & Nonnecke, B. J. (2006). Energy and protein effects on the immune system. In Ruminant physiology (pp. 455-471), Ed. Sejrsen et al., Wageningen Academic Nur Cahyo H, Niu P, Pope PB, Gimsa U, Kuhla B, Schwarm A (2026) Methane category, immune response, feed efficiency, and rumen microbial community in lactating dairy cows, J Dairy Sci, in press, https://doi.org/10.3168/jds.2025-26925 Maynez-Perez AO, Nur Cahyo H, Niu P, Aho VTE, Pope PB, Schwarm A (2026). Intricate microbiome differences observed in lactating cows across methane intensity phenotypes, ISMEcomm in review, https://www.biorxiv.org/cgi/content/short/2025.12.11.692445v1 Reiche, A.M., Sizzano, F., Siegenthaler, R., Eggerschwiler, L., Dohme-Meier F. (2025) Long-term effects of 3-nitrooxypropanol on methane production, fecal bacteria and blood immune cells in dairy cows, in abstract book EAAP – 76th Annual Meeting, Innsbruck, Austria, p 143

Effects of efficiency phenotype and forage to concentrate ratio on milk production and methane intensity in dairy cows

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Enhancing the efficiency of animal production has become increasingly important to address the rising global food requirements, lower the environmental footprint of livestock systems, and ensure economic sustainability for producers. Oxygen (O2) uptake, carbon dioxide (CO2) output, and heat production (HP) are closely linked to metabolic energy losses and have been shown to explain a large proportion of the variation in residual feed intake (RFI) in respiration chamber experiments. For this reason, indicators such as residual CO2 production (RCO2: observed CO2 – predicted CO2), residual O2 consumption, and residual heat production (RHP), calculated in a manner comparable to RFI, may serve as alternative measures of efficiency. The objective of this study was to evaluate the effects of efficiency phenotype and dietary forage to concentrate ratio (F:C) on feed intake, milk production, and methane (CH4) intensity in dairy cows using a 3-period switchback design. A 2-week pretrial was conducted with 44 Nordic red dairy cows fed a 55:45 F:C diet (F55). Residual heat production was calculated from gas exchange measurements using the GreenFeed system. Cows were ranked by RHP, and the 16 cows with the most negative values were classified as efficient, whereas the 16 cows with the most positive values were classified as inefficient. Residual heat production differed between efficiency phenotypes, averaging −9.7 ± 1.37 in efficient cows and 10.8 ± 0.68 in inefficient cows (mean ± SEM). Cows were then blocked by parity, RHP, days in milk and energy corrected milk (ECM). Within each efficiency group, 8 cows were assigned to F55 and 8 to a 70:30 (F70) F:C diet. Each period lasted 28 d; cows received the same diet in periods 1 and 3 and the alternate diet in period 2. Data were analyzed using a mixed model in SAS including fixed effects of efficiency group, diet, period, sequence, and carryover (previous diet), with block nested within efficiency group and cow nested within block as random effects. Dry matter intake did not differ between efficient and inefficient cows (21.6 vs. 20.4 kg/d; P = 0.31). Efficient cows produced more milk (31.9 vs. 24.8 kg/d; P < 0.01) and more ECM (36.6 vs. 31.3 kg/d; P < 0.01) than inefficient cows. Methane intensity (g CH4/kg ECM) was lower in efficient than inefficient cows (10.3 vs. 13.6; P < 0.01). Methane yield (g CH4/DMI) was lower for the efficient cows compared to inefficient cows (17.4 vs. 20.7; P < 0.01). The efficiency phenotype was consistent across diets, with efficient cows producing more milk, less CH4 per unit of ECM and DMI under both F:C ratios (F70 and F55). Keywords: residual heat production; feed efficiency; enteric methane; energy-corrected milk; GreenFeed system; forage to concentrate ratio

Effect of supplementing lactating dairy cows diet with an Asparagopsis taxiformis based product on enteric gaseous emission, lactation performance, and active compound milk residues

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Seaweed feed additives, particularly Asparagopsis taxiformis (AT) and armata, are of great interest due to their mode of action by directly blocking a key step of methanogenesis in the rumen. We hypothesized that the AT-based feed additive Brominata™ will reduce enteric methane (CH4) emissions in lactating dairy cows without affecting their performance or health, and that bromoform and its metabolites residues in milk will remain below levels of consumer health concern. Forty Holstein cows were enrolled in a randomized complete block design with repeated measures per cow over a 14-wk period, including 2-wk covariate and 12-wk of treatment. The study comprised two experimental diets: (1) a control diet; and (2) a control diet supplemented with Brominata™ at 0.45% inclusion rate on dry matter (DM) basis. Individual feed intake was recorded using automatic feed bins (RIC System, Hokofarm). Milk yield was recorded automatically at every milking (VMS™, DeLaval). Enteric gaseous emissions were measured using GreenFeed systems (C-Lock). Milk samples were collected bi-weekly to determine lactose, fat, and protein levels. Individual milk samples at wk6, -10, and -14 of the experiment were taken to measure bromoform, bromine, bromide and iodine concentration. Supplementation with Brominata™ reduced CH4 production by 20% ($p< 0.05$). CH4 yield (adjusted for DM intake) and intensity (adjusted for energy-corrected milk (ECM)) dropped by 19 and 26%, respectively, ($p<0.05$). Hydrogen production increased more than 2-fold in the treatment group compared to the control ($p< 0.05$). Dry matter intake was not significantly different between the experimental groups ($p= 0.09$). Milk yield and ECM were significantly higher by 5 and 8%, respectively, in animals supplemented with Brominata™ throughout the period of supplementation ($p< 0.05$). Feed efficiency, considering ECM, increased by 6.5% in the treatment group compared to the control ($p< 0.05$). Although milk protein and lactose concentrations were not affected by supplementation with Brominata™, milk fat concentration in treatment group increased by 8% ($p< 0.05$). Residual bromoform concentration in the milk was not significantly different between both groups, however bromine and bromide concentrations in the milk were significantly higher by 161% in the treatment group ($p< 0.05$). Although there are no health concerns from exposure via milk alone for these metabolites in any of the target consumer groups, iodine levels in feed premix need additional attention, as inclusion of Brominata™ increased the iodine concentration in milk by 68% ($p< 0.05$). The effective dietary inclusion rate of the additive did not lead to adverse effects on animal health and welfare. In conclusion, Brominata™ was able to mitigate the CH4 emission in lactating dairy cows without an adverse effect on animal health or posing consumer safety risks. The positive impact of Brominata™ on milk yield, ECM and feed efficiency merits further investigation.

Effect of a dietary cinnamaldehyde–eugenol–capsicum oleoresin blend on production and methane emissions in Holstein lactating cows

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Enteric methane emissions from ruminants are a major contributor to agricultural greenhouse gases, and despite phytogenic feed additives being proposed as mitigation strategies, reported responses in dairy systems remain variable. The objective of this study was to evaluate a dietary blend of cinnamaldehyde, eugenol, and capsicum oleoresin on production, feed efficiency, and enteric methane emissions in mid-lactation Holstein cows. Forty-two lactating cows were assigned to two treatments (n = 21 each): a control diet (Control) based on maize silage, grass silage, and concentrate blend, or the same diet supplemented with 1.2 g/cow/d of the XTract Ruminant X60-7065 essential blend (XTract). Cows were group-housed but individually fed using Calan gates (Calan Broadbent Feeding System; Calan Gates, American Calan; NH, USA); dry matter intake was recorded weekly, milk yield twice daily, and weekly milk samples were analysed for solids composition. Methane emissions were measured daily using the GreenFeed system (C-Lock Inc., Rapid City, SD). The feeding trial lasted 84 days, following a 2-week covariate period. Data were analysed using a repeated-measures linear mixed effects model including treatment, week, and the treatment × week interaction as fixed effects. Parity, days in milk, and the coefficient of variations for DMI and bodyweight were included as additional covariates. Cow nested within block was fitted as a random effect, and week was specified as the repeated measurement. Tukey's honestly significant difference test was used for pairwise comparisons when the effect of the fixed factors was significant ($P < 0.05$). Milk yield and feed efficiency did not differ between treatments ($P > 0.05$). XTract Ruminant X60-7065 supplementation reduced milk urea concentrations by 9.3% ($P = 0.004$; from 291 to 264 mg/L), while fat, protein, casein, lactose, and fatty acid profile were unaffected overall ($P > 0.05$). A treatment × week interaction was observed for the concentrations of milk protein ($P = 0.042$) and casein ($P = 0.037$), with higher values in XTract Ruminant X60-7065 group in Week 3 (+6.4% and +5.1%), but the difference was not statistically significant during the rest of the weeks. Methane production (g/d), yield (g/kg DMI), and intensity (g/kg milk yield and energy-corrected milk) were numerically reduced by 3.2%, 5.6%, 4.0%, and 4.5%, respectively, but the effect was not statistically significant ($P > 0.05$). However, a significant treatment × week interaction ($P = 0.049$) indicated lower methane production in the XTract Ruminant X60-7065 group (–5.2 to –7.3%) in Weeks 6–7 of the experimental period. The numerical reductions in methane emissions in the present study fell outside the statistical power threshold to detect significant effects in methane output (approximately 13%). Overall, these findings suggest that under the dietary and management conditions applied, the phytogenic blend had modest effects on production parameters and methane emissions. The reduction in milk urea and the higher milk protein and casein concentrations in parts of the study may indicate an improved nitrogen use efficiency; this warrants further investigation to include direct measurements of nitrogen balance (intake and excretion in faeces and urine), rumen ammonia concentration, and microbial protein synthesis, to confirm and clarify the biological mechanism behind this response.

Do cows rank similar in methane yield in first and second lactation?

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Dairy cows vary individually in methane (CH4) yield (CH4/dry matter intake (DMI)) and in related traits. Identification of the phenotypic traits related to variation in CH4 yield is essential for proper use of this variation to decrease CH4 emission from dairy production, both by management and by breeding. The aim of the present study was to examine whether the rankings in CH4 yield and correlated traits measured in first lactation were repeated in second lactation. Forty-eight 2nd parity cows, which at experiment onset were 99 ± 54 (mean ± SD) days in milk, were enrolled in a 3×3 Latin square design with 3-week periods and 3 treatments: Control, 3-NOP (Bovaer, 60 mg 3-NOP/kg DM) and Nitrate (Silvair, 8.5 g nitrate/kg DM). Diet composition was (g/kg DM): 262 grass-clover silage, 228 maize silage, 221 rapeseed meal, 146 barley, 86 beet pulp, 19 minerals and vitamins, and 38 concentrate mix offered in GreenFeed. Feed intake, milk yield and composition, CH4 emission (GreenFeed), and rumen fluid VFA composition (Ruminator samples) were measured in the last week of each period. All 48 cows had already been enrolled in 1st parity in a larger (201 cows) experiment with similar design, diets, treatments, and recordings, though cows were enrolled continuously 2–3 weeks after calving in the 1st parity experiment. Both experiments were analysed with mixed models with treatment and period as fixed and cow as random effects, and random cow effects (RCE) were extracted as estimates of each cow's phenotype. Forty-four cows were used for the calculations in the present study. The RCE from the two experiments (1st and 2nd parity) were correlated using Pearson correlations. Correlations between phenotypes (RCE) for CH4 yield (g/kg DMI) in 1st and 2nd parity were 0.48 ($P = 0.001$). Further, traits correlating to CH4 yield in 1st parity were examined for correlations between 1st and 2nd parity. Traits correlating positively with CH4 yield in 1st parity, milk fat yield (kg/d, $r = 0.55$, $P < 0.001$) and rumen acetate (mol/100 mol, $r = 0.48$, $P < 0.001$) also correlated positively between 1st and 2nd parity. Traits correlating negatively with CH4 yield in 1st parity, milk yield (kg/d, $r = 0.71$, $P < 0.001$), energy corrected milk yield (kg/d, $r = 0.66$, $P < 0.001$), rumen valerate proportion (mol/100 mol, $r = 0.60$, $P < 0.001$), rumen propionate proportion (mol/100 mol, $r = 0.54$, $P < 0.001$), and DMI (kg/d, $r = 0.52$, $P < 0.001$), likewise correlated positively between 1st and 2nd parity. According to the correlations, cows' CH4 yield ranked similarly in 1st and 2nd parity. The same applied to several other traits identified to correlate positively or negatively with CH4 yield in 1st lactation. This indicates, that both CH4 yield and several traits correlated to CH4 yield, such as well-known phenotypes as feed intake and milk production, but also phenotypes as rumen volatile fatty acid fermentation pattern correlate positively between 1st and 2nd parity and thereby could be used as supporting traits for cows' CH4 yield.

Effect of rice bran supplementation on nutrient utilization and methane emissions of beef heifers grazing native pasture

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Beef cattle raised on native grasslands experience strong seasonal fluctuations in forage supply, leading to inconsistent growth of replacement heifers. Winter supplementation with full fat rice bran is commonly used in rice-growing areas to improve performance, yet the effects of this practice on enteric methane emissions remain unclear. This study evaluated the effects of rice bran supplementation on nutrient intake, digestion, N and water balance, and methane emissions in beef heifers grazing native pastures during winter. The experiment was conducted at Palo a Pique Experimental Unit of INIA, Treinta y Tres, Uruguay (33ºS and 56ºW) during the winter season (i.e. July to September). Forty-eight Aberdeen Angus × Hereford heifers (355 ± 6,4 kg BW) were blocked by BW and randomly assigned to one of two treatments: (i) native pasture (NP, n = 24), and (ii) native pasture plus rice bran supplementation at 0.7% of BW (SUP, n = 24). All heifers grazed together, and SUP received their supplement once daily. Measurements were conducted during two consecutive periods (40 days) in which animals remained under the same treatment. Methane emissions were measured using the GreenFeed® system. To estimate intake and digestibility of nutrients, the animals (n = 10/treatment) were allocated in daily individual paddocks (100 m2) with a daily forage allowance of approximately 30 kg of DM per animal for 5 consecutive days in each of the two periods. The pasture intake was estimated individually as the difference between the pre- and post-grazing forage mass. Apparent total-tract nutrient digestibility was determined by collecting total feces deposited each day in each grazed paddock. To estimate the voluntary water intake, the amount of water offered and left water orts from each per animal was measured using a individual bins (capacity 100 L). Daily methane emissions did not differ between treatments. Supplementation increased nutrient DM intake ($P < 0.01$) through a positive associative effect and improved intake and digestibility of organic matter, crude protein, ether extract, and non-structural carbohydrates ($P<0.01$). Nitrogen intake and faecal nitrogen excretion increased with supplementation ($P<0.01$), whereas urinary nitrogen excretion decreased ($P<0.01$), suggesting greater absorbed more favorable nitrogen incorporated in body tissues partitioning. Voluntary water intake was unaffected. Rice bran supplementation enhanced nutrient intake without increasing absolute emissions, demonstrating that moderate lipid-rich supplementation is an effective strategy to improve the efficiency and environmental sustainability of in rangeland systems.

Methane emission response to feed supplements differs among cows

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Feeding strategies in dairy cows may be used to mitigate methane emission and reduce the impact of dairy farming on global warming. Feed supplements, added to the basal ration, can reduce methane emissions through different pathways that interfere with methanogenesis in the rumen. Given that methane emission itself is highly variable among cows, the efficacy of various supplements may also differ among animals. Therefore, our aim was to evaluate between-cow variation in the methane response to three different supplements and explore what cow characteristics predict the response. Thirty-two Holstein Friesian dairy cows each received four treatments for four weeks in a Latin-square design. Treatments included three different supplements, as well as a control treatment without any supplement. Supplement 1 consisted of a mixture of fats, supplement 2 of a blend of essential oils, and supplement 3 of a blend of seaweeds. Methane concentration and airflow were measured in a Greenfeed station and converted to individual production (g) per cow per day. Cow characteristics (27 in total), including various variables describing cow condition and behaviour, were also measured by a reticulum bolus and a neck sensor. Each of the variables was extracted for the last week of each treatment, assuming that the cow had adjusted to the treatment by then. The response in methane production to each of the supplements was extracted by subtracting the methane production from the production in the control treatment. Stepwise linear regression models were developed for each supplement to predict the methane production response to the supplement using cow characteristics. Cow characteristics were added to or removed from the model using stepwise forward and backward regression, based on partial F-tests evaluating the change in residual sum of squares (model entry criterion $p<.05$; removal criterion $p>.10$). Methane emission response to each of the three supplements varied greatly among cows, and the supplement optimal for methane reduction differed across cows. For supplement 1, methane reduction was stronger for cows with a higher minimum reticulum pH and longer rumination time in the control treatment (full model adj. $R^2=.23$, $p=.02$). For supplement 2, reduction was stronger for cows that drank smaller portions of water more frequently (full model adj. $R^2=.37$, $p<.001$). For supplement 3, reduction was stronger for cows with a higher dry matter intake and that drank smaller portions of water (full model adj. $R^2=.33$, $p<.01$). In conclusion, individual response to feed supplements for methane mitigation differed among cows. Individual cow characteristics, including reticulum pH, water drinking behaviour and dry matter intake, were associated with the individual cow response to the methane-mitigating feed supplements. These results suggest that each of the supplements targets methane emission through different physiological mechanisms. Fat supplements are known to especially target fiber-digesting microbes that thrive under healthy rumen pH conditions, which might explain their greater effectiveness in cows with a higher minimum reticulorumen pH. Essential oils and seaweeds seem to perform better with a steady rumen passage rate, as caused by frequent small drinking bouts. However, physiological mechanisms need further exploration, which we plan to do by including results from blood and milk sampling. The developed models require validation on a larger dataset. Ultimately, they have the potential to enable precision supplement tuning, contributing to reducing methane emissions in dairy cows.

Dietary sweet chestnut tannins decrease diarrhea incidence and litter nitrogenous gas emissions in pre-weaning holstein calves

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Tannins are plant bioactive compounds with the potential to enhance animal health and reduce the environmental footprint of livestock production. This study investigated the effects of supplementing hydrolysable tannins from sweet chestnut (Castanea sativa Mill.) on the growth performance, intestinal health, and nitrous oxide (N_2O) and ammonia (NH_3) emissions from the litter of Holstein calves. Twenty-four pre-weaning female Holstein calves were assigned to two experimental groups for a 56-day trial: a control group (CTRL) fed with milk replacer, and a treatment group (TRT) fed milk replacer supplemented with 10 g of tannins. Body weight (BW) and rectal temperature (RT) were recorded at d 0, 7, 14, 21, 28, 35, 42, 49, and 56. Milk intake (MI), hay intake (HI), average daily feed intake (ADFI) of solid feed, average daily gain (ADG), and fecal and clinical scores were recorded daily. Fecal samples were collected at d 0, d 28, and d 56 to determine total bacterial counts, coliforms, and Lactobacillus spp. Blood samples were collected at d 0 and d 56 for metabolic profile analysis. After the in vivo trial, litter (feces, urine, and straw) was collected from individual boxes and pooled into dynamic chambers to evaluate emissions between the two groups for an additional 49 days. Emissions of nitrous oxide (N_2O), and NH_3 (mg/g/h) were monitored by Gasmet-GT5000, and chemical analyses were performed at d 0 and d 49. Over the 56-day trial, no significant differences were observed between TRT and CTRL groups for MI, HI, BW, ADG, and RT ($P>0.05$). However, tannin supplementation significantly reduced ADFI ($P<0.01$) without compromising growth performance. The TRT group showed significantly higher frequency of normal fecal consistency (FS 0-1 score; $P<0.01$) and reduced diarrhea incidence (FS 2-3 score). Fecal color analysis revealed more brown feces in TRT versus more yellow feces in CTRL ($P<0.01$). Clinical scores and remained unaffected by treatment, and blood metabolic parameters remained within normal physiological ranges. Bacterial counts showed no group differences between groups, total bacteria and coliforms remained stable, while Lactobacillus spp. increased in both groups over time. Tannin supplementation significantly influenced litter emissions, reducing NH_3 (-58%, $P<0.01$) and N_2O (-60%%, $P<0.01$) emission. Particularly, NH_3 emissions were lower at d 0 and 1 ($P<0.01$), while N_2O emissions decreased at d 1, 2, and 5 in the TRT group. Litter temperature increased in the TRT group at d 0, d 1, and d 2 ($P<0.05$). Chemical analysis indicated lower total solids, ash, and total carbon in TRT litter at day 49, consistent with observed emission reductions. Furthermore, tannins reduced both ammonia and nitrous oxide emissions from calf litter, likely by modulating organic matter fermentation and enhancing litter stabilization. These findings highlight tannins as a promising feed additive to improve calf health while simultaneously reducing the environmental impact of livestock operations.

Rumen microbial adaptation time following dietary shifts and diurnal variation post-feeding

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Diet strongly shapes the rumen microbiome. While fermentation parameters such as volatile fatty acids and dry matter (DM) intake generally stabilize within two weeks after dietary change[1], the adaptation period required for the microbial community itself remains poorly characterized. In addition, diurnal fluctuations associated with feeding may influence microbial composition, potentially confounding treatment effects in nutritional intervention studies. This study aimed to determine (i) whether a two-week adaptation period is sufficient for rumen microbial stabilization and (ii) the impact of diurnal variation on microbial ecology. We hypothesized that the rumen microbiome stabilizes within two weeks after a dietary shift and varies across the day. Eight rumen-cannulated, second-parity Holstein cows were housed individually and fed two experimental diets differing in roughage proportion (LOW: 35% DM; HIGH: 63% DM) in a crossover design, with each diet fed for three weeks. In the first period, half of the cows received LOW and half HIGH, and in the second period, cows were switched to the opposite diet. Cows had ad libitum access to feed and water and were offered fresh feed at 08.00 h. To assess microbial adaptation, rumen grab samples were collected once before the first diet shift and weekly for three weeks in each period (N=56). To evaluate diurnal variation, additional samples were collected at five time points within a day (06:00, 09:00, 11:00, 14:00, and 22:00 h) at the end of week three of each period (N=80). All samples underwent shotgun metagenome sequencing, with a subset of the diurnal samples subjected to metatranscriptome sequencing (N=48). Analyses included microbial composition, diversity metrics, differential abundance, and functional profiling. The largest shifts in microbial composition occurred within the first week following dietary change. However, microbial composition did not fully stabilize within two weeks. Microbial richness declined immediately after diet shift, with stronger reductions under LOW. Richness and Shannon diversity remained below baseline during LOW feeding, whereas cows receiving HIGH showed rapid recovery and a progressive increase, reaching highest richness and diversity three weeks. Across all sampling weeks, alpha diversities remained consistently higher in HIGH than LOW. Diurnal patterns revealed short-term dynamics in response to offering fresh feed. Richness and Shannon diversity increased after feeding, peaked three hours post-feeding, and returned to pre-feeding levels. Although similar patterns were observed under both diets, statistically supported was detected only under LOW. Diurnal compositional changes were detected but with a small effect size, primarily driven by pre- and early post-feeding samples (2 h before vs. 1 h after feeding), while samples within the six-hour post-feeding window remained relatively stable. Diurnal sampling time explained substantially more variation in microbial activity than composition, indicating stronger temporal effects on microbial function than community structure. Findings indicate that both adaptation period and sampling time influence rumen microbiome measurements. Overall, these results emphasize the need for adaptation periods longer than two weeks and carefully defined sampling protocols to ensure that rumen microbiome data accurately reflect dietary effects rather than transient microbial fluctuations.

[1] Machado et al., 2016, Animal Feed Science and Technology, 222, 132–148.

Validation of global methane prediction equations for Austrian dairy cows under silage-based rations

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Accurate prediction of enteric methane (CH_4) from dairy cows is key to mitigating agriculture's climate impact. This study validated 21 international CH_4 equations using respiration chamber data from Austrian cows fed Central European silage-based diets. Data from 76 lactating cows (56 Holstein-Friesian, 20 Fleckvieh) were analyzed. Cows differed in days in milk (20–300 d), parity (1–4), body weight (550–750 kg), and milk yield (20–55 kg/d). Diets comprised grass silage (35–45% NDF in DM), hay, maize silage, and concentrates. Dry matter intake (DMI) ranged from 15–28 kg/d; measured CH_4 was 219–476 g/d. Methane was recorded in two parallel respiration chambers (Innova 1412i) at 20°C and 60% relative humidity, with 2 d adaptation and 2 d measurement. Equations ($r^2 \geq 0.65$) were classified as simple (1 parameter; e.g., Jiao et al., 2014; Ribeiro et al., 2020), moderate (2–4; e.g., Yan et al., 2009), or complex (≥5; e.g., Jentsch et al., 2007; Marumo et al., 2023). Inputs included DMI, gross energy intake (GEI), body weight, forage share, NDF, digestibility (dCP, dEE, dCF, dNFE), and milk traits. Units were standardized (Kirchgessner et al., 2014: 1 g CH_4 = 1.4 L = 55 kJ). Outliers (1.5×IQR) were removed, <5% values imputed, and daily means calculated. Models were evaluated by sum of squared errors of prediction (SSEP), error decomposition, ANOVA ($p < 0.05$), and Bland–Altman analysis. Applicability considered farm data availability. Ribeiro et al. (2020) Eq. 49 ($CH_4 = 3.35 + 0.047 \times GEI$) performed best (SSEP 0.214 g^2/d; ratio 1.0; $R^2 \approx 0.90$), followed by Ribeiro et al. (2020) Eq. 1 ($4.15 + 0.822 \times DMI$; 0.216) and Marumo et al. (2023) ($38.0 + 19.22 \times DMI$; 0.218). Simple GEI- and DMI-based models dominated. Jentsch et al. (2007) Eq. 5 performed worst (SSEP 16.998). Complex digestibility models tended to overpredict high and underpredict low emissions. Bland–Altman plots showed minor bias around 300–350 g/d, with underprediction in early lactation and overprediction at high DMI. Agreement was slightly better for Fleckvieh. ANOVA confirmed significant differences ($p < 0.01$); top models achieved 15–80× lower SSEP than the weakest. GEI and DMI were confirmed as main CH_4 drivers (Ellis et al., 2007; Marumo et al., 2023). Ribeiro et al. (2020) Eq. 49 is recommended for Austrian systems due to high accuracy, simplicity (GEI = DMI × energy density), and robustness across silage diets. DMI-based models are practical alternatives. Notably, the Brazilian equation outperformed European and North American models. These equations support inventories, feed optimization (e.g., concentrate changes reducing CH_4/kg milk by 10–20%), and integration into breeding or ration tools. Regional validation supports EU targets (40% reduction by 2030). Future work should link CH_4 and DMI prediction and test pasture systems to enable mitigation without productivity loss. Literature: Ellis et al., J Dairy Sci 2007;90:1970–1980 Jiao et al., Anim Feed Sci Technol 2014;194:1–11 Jentsch et al., Arch Tierernähr 2007;61:10–19 Kirchgessner et al., Tierärztl Umsch 2014;69:123–132 Marumo et al., Anim Sci J 2023;94:e13928 Ribeiro et al., Animal 2020;14:s2–s10 Yan et al., J Dairy Sci 2009;92:4632–4643

Hand-held laser device as a tool to monitor relative differential enteric methane emissions in dairy cows supplemented or not with a natural feed additive

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Interest in spot sampling strategies to estimate enteric methane emissions is consistently increasing. These approaches are generally less expensive, highly adaptable to different rearing systems without necessarily being linked to experimental facilities (e.g., commercial barns), and economically convenient. Meanwhile, natural feed additives are emerging as potential strategies to mitigate the environmental impact of ruminants, particularly methane emissions. In our previous in vitro study, several natural compounds, their natural-identical forms, and combinations of essential oil molecules and tannin extracts were tested. The in vitro screening included 12 individual bioactive compounds and 36 mixtures. Among these, one additive composed of tannins (quebracho) and essential oils (carvacrol, eugenol and limonene) showed promising effects in reducing methane and ruminal ammonia at a dosage of 133 mg/L of ruminal liquor for the tannin extracts and 100 mg/L for the essential oil blend. The aim of the present study was therefore to evaluate methane emissions and milk yield following supplementation of the same additive (ADD) to a control basal diet (CON) in dairy cows reared in an indoor commercial barn. A total of twenty mid-lactation Holstein-Friesian cows were included in the study and allotted to two groups of 10 cows, balanced for milk yield (20.3 ± 3.2; 21.4 ± 3.2 L), body weight (693 ± 68; 689 ± 48 kg), days in milk (145 ± 61; 145 ± 55), and parity (2.0 ± 1.1; 2.0 ± 0.9). The basal diet was a total mixed ration containing (g/kg): 920 organic matter, 140 crude protein, 30 ether extract, 180 starch, and 410 neutral detergent fiber. The additive was top-dressed and its intake was monitored until complete consumption to achieve a ruminal dosage comparable to that used in the in vitro study. The trial lasted 10 weeks, during which a hand-held laser methane detector (LMD) was used in week 0 (covariate period) and from weeks 6 to 10, following the adaptation period to the additive. Milk yield was recorded daily. Methane data for each period were derived from six spot measurements of 5 minutes each, performed over three days per week and considered representative of a 24-h period. Data were processed using a common local maximum algorithm to detect "peaks", and absolute methane production was calculated using the equation proposed by Sorg et al. (2018): CH_4 (g/d) = 150 + 2.2 × peaks (ppm × m). Statistical analysis was performed using a linear mixed model including treatment and week as fixed effects, cow as a random effect, and the corresponding baseline value of the variable as a covariate. Methane emissions varied across weeks but remained consistent between treatments. For example, at week 10, estimated methane emissions were 403.1 ± 11.8 and 412.8 ± 13.7 g/d for CON and ADD, respectively. Similarly, methane intensity (25.6 ± 1.70 g/L of milk), milk yield (16.9 ± 0.86 L/d) and fat yield (0.7 ± 0.05 kg/d) did not differ between treatments. Further studies are needed to validate the design of the spot-sampling measurements to determine whether the sampling scheme applied was sufficiently accurate to detect emissions differences between groups and, more generally, whether the natural additive itself may produce detectable effects under experimental conditions.

Effects of individual and stacked feed additives on in vitro methane production

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Reducing enteric methane (CH_4) from ruminant livestock is essential for sustainable agriculture, as it is a major greenhouse gas and a loss of dietary energy. Although individual feed additives (FAD) with anti-methanogenic potential can reduce emissions, their effects are often variable and dose-dependent, with potential impacts on rumen fermentation. Therefore, recent research has focused on stacking additives with complementary modes of action to achieve more consistent CH_4 mitigation. We hypothesised that combining FAD with distinct mechanisms would result in greater CH_4 suppression than their individual application. This study evaluated, in vitro, the individual and combined effects of 3 existing or near-to-the-market FAD: a blend of essential oils, bioflavonoids, and tannins (A), nitrate (B; an alternative hydrogen sink), and calcium peroxide (CaO2; C; an oxygen-releasing compound), on CH_4 production and rumen fermentation dynamics. Batch fermentations were conducted in 3 independent runs using perennial ryegrass silage as the substrate, incubated with buffered rumen fluid in 100 mL serum bottles at 39 °C for 48h under anaerobic conditions. Rumen fluid was collected post-slaughter from 5 donor cattle at a commercial abattoir in each run, transported to the laboratory in pre-warmed thermos flasks, strained through cheesecloth, and preserved under CO_2 to preserve anaerobic conditions before incubation. Treatments included A, B, and C at low, medium, and high doses, alongside stacked combinations AB, AC, BC, and ABC and a control diet (CON) tested in triplicates in each run. Methane in headspace gas was quantified at 4, 24, and 48h by gas chromatography and expressed as mL CH_4 per bottle and as a percentage change relative to CON. Data were analysed using SAS (PROC GLM) to assess the effects of treatment, dose, time and run. Model assumptions were verified, and Tukey's post hoc tests were applied when significant effects were detected ($p<0.05$). Calcium peroxide alone was highly effective across all doses. At 24h, CON produced 4.2 mL of CH_4, but C at the high dose produced only 0.7 mL. Similarly, at 48h, CON CH_4 averaged 4.6 mL against 0.7 mL for C-high, both a drop of ≈85%. Nitrate showed a clear dose-dependent response: at 24h the high dose reduced CH_4 from 4.2 to 2.9 mL (≈ -32%), and at 48h from 4.6 to 2.9 mL (≈ -38%). In contrast, A produced little or no mitigation at 24h and only modest reductions at 48h (≈7-15%). When stacked, strong mitigation was observed only when CaO2 was included. At 24h, CON produced 4.2 mL of CH_4, while AC, BC, and ABC all produced ≈0.6 mL, all reductions of approximately 85% (≈ -3.6 mL). At 48h, CON CH_4 averaged 5.1 mL, while AC, BC, and ABC treatments produced only 0.63-0.58 mL, representing reductions of ≈4.5 mL (≈ -89%). By contrast, the AB blend provided minimal mitigation (≈ -8% at 48h). These results do not support additive or synergistic effects beyond those driven by CaO2. Treatments containing CaO2 also consistently lowered headspace pressure and reduced ammonia accumulation over time, indicating altered fermentation pathways. Transient increases in the acetate:propionate ratio were observed during early fermentation but diminished by 48h, suggesting microbial adaptation. In conclusion, the initial hypothesis was only partially supported, as CaO2 was the main driver of CH_4 reduction and stacking with nitrate or compound A maintained but did not enhance it. These findings support further in vivo evaluation of CaO2-based FAD strategies for practical CH_4 mitigation in ruminant diets.

Effects of garlic extract-attapulgite hybrid mixture on performance and enteric methane production in Hanwoo steers

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In vivo evaluation of methane (CH_4)-reducing feed additives is essential for the practical implementation of effective enteric CH_4 mitigation strategies in Korea. An in vivo trial was conducted using twelve Hanwoo steers (395 ± 9 kg), randomly allocated to three groups of four animals each. Steers were fed a commercial concentrate and rice straw hay (65:35) either without supplementation (CON) or supplemented with an additive containing garlic extract-attapulgite hybrid mixture (GAA) at 1.0% of dry matter intake (DMI) for 52 days in a completely randomized design. Animals were adapted to the experimental diets for 43 days in pens, followed by a 4-day adaptation period in open-circuit whole-body respiratory chambers (RC). Methane production was subsequently measured for 3 consecutive days using the RC system, and rumen fluid samples were collected on the final day of the trial. Supplementation with GAA reduced ($p < 0.05$) CH_4 production by 11.3% (140.4 vs. 124.5 g/d) and CH_4 yield by 8.2% (20.2 vs. 18.6 g/kg DMI) compared with CON. No negative effects of GAA supplementation on feed intake or animal performance were observed. Ruminal pH, ammonia concentration, and total volatile fatty acid concentration were not affected by treatment ($p > 0.05$); however, GAA supplementation tended to decrease ($p = 0.084$) isovalerate proportion. Overall, GAA supplementation reduced enteric CH_4 emissions without adversely affecting animal performance. Further long-term studies are warranted to evaluate the persistency of its CH_4-mitigating effects.

Effects of low levels of nitrate supplementation on milk and methane production in lactating dairy cows

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Supplementing nitrate is a well-recognized strategy to reduce enteric methane (CH4) emissions in dairy cows. Most of the previously published studies evaluated nitrate doses of 10 and 20 g/kg of DM, with very limited information available on the effects of lower inclusion levels on methane and performance. The objective of this experiment was to evaluate animal performance and methane production in lactating dairy cows when supplementing diets with lower than the typically studied doses of nitrate. Twelve Holstein cows (averaging 40.2 ± 19.0 kg/d in milk; and 143 ± 89 DIM; mean ± SD) were used in a 4 x 4 Latin square design experiment with four periods of 28 days each (21 d for adaptation and 7 d for data collection). Cows were individually fed a corn silage and alfalfa haylage-based TMR (58% forage, 16.6% CP, 30.5% NDF, 25.2% starch, and 2.6% fat DM basis). Treatments were: 0 (CON), 2.5 (2.5NIT), 5 (5NIT), and 7.5 (7.5NIT) g/kg of DM as nitrate (calcium ammonium nitrate, SilvAirTM, Cargill Inc.). Nitrate replaced urea and calcium carbonate to maintain N and Ca similar among dietary treatments. Gas production was measured using the GreenFeed system (C-Lock Inc.). Each cow was brought to the GreenFeed eight times over three days. The visits were spaced nine hours apart, resulting in eight samples per cow in each period, distributed every three hours throughout the day. Data were analyzed with the lmer package in R with a model that contained the fixed effect of diet and the random effects of period, square, and experimental unit. For repeated measurements throughout the day, treatment, time, and their interaction were also included in the model. Contrasts were used to evaluate the linear and quadratic effects of nitrate dose as well as the overall effect of nitrate. Means in the subsequent text are reported in the following order: CON, 2.5NIT, 5NIT, and 7.5NIT. There was no significant treatment by time interaction for any of the gas measurements. Increasing nitrate supplementation in isonitrogenous diets decreased ($P < 0.01$) methane production linearly (455, 414, 411, and 369 g/d; SEM = 39) with a reduction of -9.02, -9.74, and 18.9% from CON. Production of H2 increased linearly ($P < 0.01$) by 48.7, 70.9, and, 125% compared with CON. Nitrate supplementation linearly ($P < 0.05$) decreased methane yield (17.1, 15.8, 15.8, and 14.1 g of CH4/kg DMI; SEM = 1.0) and methane intensity (10.8, 10.2, 10.0, and 9.22 g of CH4/kg energy-corrected milk; SEM = 0.62). Dry matter intake linearly decreased ($P = 0.01$) when urea was replaced by nitrate (26.5, 25.9, 25.5, and 24.3 kg/d; SEM = 2.1). However, nitrate supplementation did not affect ($P = 0.87$) milk yield (36.8 ± 2.7) and therefore, nitrate supplementation tended to increase ($P = 0.06$) feed efficiency linearly (1.42, 1.43, 1.47, and 1.45; SEM = 0.08) Nitrate supplementation linearly decreased ($P < 0.05$) milk fat content (4.26, 4.16, 4.11, and 4.06%; SEM = 0.18) and milk protein content (3.49, 3.43, 3.43, and 3.42%; SEM = 0.15) and tended to decrease ($P = 0.08$) milk fat yield (1.56, 1.50, 1.50, and 1.45 kg/d; SEM = 0.09) but did not affect milk protein yield (1.26 ± 0.10 kg/d). Treatments did not affect energy-corrected milk yield (41.2 ± 2.84 kg/d). Overall, this study enhances the evidence that nitrate doses of 2.5, 5, and 7.5 g/kg of DM are effective in reducing methane production, yield, and intensity in lactating dairy cows. These results will help to select the most appropriate dose, aligning methane-reduction targets with the economic context of diverse milk production systems.

Mitigation of methane emissions and modulation of rumen microbiome by Asparagopsis-based feed additives in Hanwoo steers

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Asparagopsis, a red seaweed rich in bromoform, has shown strong methane-reducing potential in ruminants, yet its application in Hanwoo cattle remains underexplored. Therefore, this study evaluated the effects of an Asparagopsis-based feed additive on methane (CH4) emissions, rumen microbial composition, and functional profiles in Hanwoo steers. Twelve Hanwoo steers (625.92 ± 64.14 kg and approximately 21 months old) were used in a replicated 3 × 3 Latin square design with 3 treatments and 3 periods of 21 days. Steers received a basal diet (9.6 kg of commercial concentrate feed and 2.4 kg of forage) with one of three additives: no additive (CON), 0.5% Asparagopsis-based feed additive (AS; CH4 Global, Inc.) containing 4 mg bromoform/g dry matter (DM) (AS4), or 0.5% AS additive containing 7 mg bromoform/g DM (AS7). Enteric CH4 emissions were measured using the GreenFeed system. Methane emission was expressed as CH4 production (g/d), yield (g/kg DMI), and intensity (g/kg $BW_{0.75}$). Shotgun metagenomic sequencing was employed to characterize the microbial composition and functional profile of the rumen microbiome. Results showed that Asparagopsis supplementation markedly reduced CH4 emissions. Specifically, steers supplemented with AS4 and AS7 produced 37.25% and 45.88% less CH4, respectively, than the CON group ($p < 0.001$). Moreover, CH4 yield decreased by 38.06% and 44.78% ($p < 0.001$), while CH4 intensity was reduced by 36.76% and 44.85%, in the AS4 and AS7 groups, respectively ($p < 0.01$). Shotgun metagenomic sequencing revealed a total of 23 phyla, 42 classes, 87 orders, 173 families, and 410 microbial genera in the rumen samples. The bacterial phyla Bacteroidetes and Bacillota, along with the archaeal phylum Methanobacteriota, were the most abundant taxa observed. Prevotella was the dominant bacterial genus, showing a higher relative abundance in the rumen of steers from the AS4 group. Methanobrevibacter was the predominant methanogenic genus, accounting for approximately 13% of the rumen metagenome, and its relative abundance was significantly higher in steers supplemented with Asparagopsis-based feed additives. Six classes of carbohydrate-active enzymes (CAZymes), AA, CBM, CE, GH, GT, and PL, were identified in the metagenome, with GH and GT being the most abundant, accounting for approximately 85% of the total CAZymes. AS supplementation increased the abundance of GH13, GH3, GH31, CBM48, GH5, GT35, GH9, GH77, GT51, CE10, GH43, and GH95 compared to the CON group. Notably, GH3 and GH95 were enriched in the AS7 group, while CE10 levels were higher in both AS4 and AS7 ($p < 0.05$). KEGG pathway analysis revealed that the functions of the rumen microbiota were associated with carbohydrate, amino acid, energy, and nucleotide metabolism. The relative abundance of the CH4 metabolism pathway (ko00680) was lower in the AS4 group compared with the CON and AS7 groups. Overall, supplementation with Asparagopsis-based feed additives at 0.5% inclusion level effectively reduced CH4 emissions in Hanwoo steers by up to 45%. These reductions were accompanied by shifts toward higher Prevotella abundance and lower CH4 metabolism pathway activity. These findings highlight the potential of Asparagopsis as a practical CH4 mitigation strategy for Hanwoo production without compromising rumen microbial diversity or key functional profiles.

Seasonal variability in the response of enteric methane emissions to 3-nitrooxypropanol in dairy cattle

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Mitigation of enteric methane (CH_4) emissions through inhibition of ruminal methanogenesis is a promising strategy to reduce the climate footprint of ruminant production. This study evaluated the seasonal response of methane emissions, hydrogen dynamics and metabolic indicators to 3-nitrooxypropanol (3-NOP) in dairy cattle. Gas exchange was measured using GreenFeed systems (C-Lock) in 24 animals (Control n = 12; 3-NOP n = 12) during two experimental periods (winter and summer). Emissions of CH_4, CO_2, O_2 and H_2 were recorded and used to derive methane yield (Ym), respiratory quotient (RQ) and heat production (HP). During winter, 3-NOP significantly reduced methane emissions by 27.7% (465 vs 336 g CH_4 d^{-1}; p = 0.00022). Hydrogen emissions increased markedly (0.84 vs 2.85 g d^{-1}; +239%; $p < 10^{-128}$), while CO_2 (10.7 kg d^{-1}) and O_2 consumption (~7.7 kg d^{-1}) were not affected. Metabolic indicators remained stable, with RQ close to unity (1.01 vs 1.02; p = 0.53) and heat production approximately 27 Mcal d^{-1} (p = 0.75), indicating that overall metabolic rate and substrate oxidation were unchanged. Methane intensity metrics decreased significantly, including CH_4/CO_2 (p = 0.00085), CH_4/O_2 (p = 0.0013) and CH_4/heat production (p = 0.00116). Methane yield (Ym) declined from ~7.1% to ~5.1% of gross energy intake, representing an approximate 28% mitigation. In contrast, during summer, methane emissions were higher in the 3-NOP group (367 vs 283 g CH_4 d^{-1}; +30%; p = 0.0037) and hydrogen emissions also increased (0.997 vs 1.43 g d^{-1}; $p = 3.1 \times 10^{-8}$). Methane intensity indicators, including CH_4/CO_2, CH_4/heat production and Ym, were also higher in the treated animals. Metabolic indicators showed a different pattern compared with winter, with lower heat production (p = 0.00052) and higher respiratory quotient (p = 0.00022), suggesting a shift toward carbohydrate-derived fermentation pathways. Methane production remained strongly coupled with CO_2 emissions ($R^2 = 0.77$; p = 0.005), indicating that methanogenesis continued to function as the dominant hydrogen sink. Hydrogen balance calculations further highlighted the seasonal contrast. In winter, methane reduction (−129 g CH_4 d^{-1}) corresponded to approximately 65 g H_2 equivalents, of which only ~3.1% appeared as molecular hydrogen while ~96.9% were redirected to alternative metabolic pathways. In summer, the additional hydrogen produced was almost entirely captured by methanogenesis, with only ~1% emitted as H2 gas. Overall, the study demonstrates effective methane inhibition by 3-NOP during winter without changes in host metabolic rate, whereas the summer period showed higher methane production and increased methane intensity, suggesting a potential seasonal modulation of rumen hydrogen metabolism. Because this response contrasts with the expected inhibitory effect of 3-NOP, additional summer measurements are required to confirm the observed seasonal pattern and identify the underlying mechanisms regulating hydrogen partitioning in the rumen.

Bovaer separation by feed particle size at two inclusion doses in dairy cows mixed rations

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The additive Bovaer can be used to reduce enteric methane from cattle at a dose of min. 60 mg 3-nitrooxypropanol (3-NOP) per kg DM. This study investigated whether Bovaer can separate by feed particle size, which might result in sorting for or against the additive when the mixed ration is fed. During a trial two partial mixed rations (PMR) with either 62 or 93 mg 3-NOP/kg of DM were fed to lactating dairy cows (60BOV and 90BOV, respectively). The PMR were formulated at 54:46 forage to concentrate ratio (on DM basis) and included maize silage (70% DM of forage), grass-clover silage, barley, rapeseed cake, dried beet pulp (crushed pellets), and ingredients for micronutrients. Samples of the PMR were taken on Tuesdays on week 3, 6, and 9 during the trial. On each day, the samples were mixed and further split in 3 sub-samples. Each sub-sample was processed in a Penn State Particle Separator (PSPS) with sieves of 19, 8, and 1.18 mm, where each sub-sample had its own run in the PSPS. The PSPS was mounted on a rotary shaker set at 200 rpm for 20 s. In each run, the weight of the sub-sample before sieving and then for each sieve fraction were recorded. The fractions obtained by the PSPS were pooled between the 3 runs by sieve and Bovaer dose. Then, the pooled sample was split in 2 and used for DM analysis in a forced air oven at 60°C or transferred to a 500 mL HDPE container, and frozen for shipment and analysis of 3-NOP by high performance liquid chromatography. The average weight fraction of the original feed samples recovered in each sieve were calculated. Statistical analyses were performed in R using GLMMs. The first model tested the difference in DM concentration (DM%) between fractions, with DM% by particle size as a dependent variable, particle size as fixed effect, and week as random effect. One outlier was dropped from this model. The second model tested the difference in 3-NOP concentration by particle size, and it was similar to the previous model, but it included the additional effect of 3-NOP dose (60BOV or 90BOV), and its interaction with the particle size. The particle size effect consisted of 4 levels: >19 mm, ≤19 and >8 mm, ≤8 and >1.18 mm, and ≤1.18 mm. Across the 3 weeks, the average fresh weight of the starting feed sample for a single run in the PSPS was 693±62.1 g for 60BOV and 728±60.8 for 90BOV. The DM concentration was 38.8, 40.0, 44.5, 46.6% from largest to smallest particles size, with max SEM of 1.28%. The 3-NOP concentration in DM was unaffected by the feed particle size and no interaction was found between particle size and 3-NOP concentration. The concentration of 3-NOP was different between the 2 treatments ($P<0.001$) with estimated means and SEM of 57.2±10.3 (60BOV) and 84.9±15.3 (90BOV) mg 3-NOP/kg DM. The SEM based CV for 3-NOP concentration was over 15%, against the SEM based CV for DM% below 5%. The estimated means concentration of 3-NOP was 58.8, 57.6, 55.1, and 57.6 mg 3-NOP/kg DM for 60BOV, and 84.8, 73.8, 83.2, and 99.7 mg 3-NOP/kg DM for 90BOV. Overall, the distribution of 3-NOP between particle size was homogeneous. Also, the numeric difference in 3-NOP concentration between particle sizes at 60 mg 3-NOP/kg DM, which is the legally allowed minimum dose in the EU, was negligible. In conclusion, no differences in concentration of 3-NOP in different particle size fractions of a mixed ration were found when 3-NOP was dosed at either 63 or 92 mg 3-NOP/kg DM, indicating that the risk for animal variation in 3-NOP uptake due to sorting is minor.

In vitro evaluation of the effect of plant extracts on methane production and fiber degradation in a rumen ecosystem

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Methane (CH4) emitted by ruminants is the main cause of greenhouse gas emissions from livestock farming. Methanogenesis by Archaea depends on H2 and CO_2 production by rumen microbiota, which is intricately linked to the degradation of plant fibers in the rumen. Reducing methanogenesis without impairing fibrolysis is crucial to maintain production and health of cattle. A growing body of evidence suggests that certain plant extracts (PE) can modulate rumen fermentation and mitigate enteric CH_4 emissions (Arndt et al, 2022). The first objective of this in vitro trial was to compare the effects of 2 new mustard extracts (ME) differing in composition to other plant extracts documented to alter CH_4 production in the bovine rumen ecosystem at the tested concentrations (Honan et al. 2020). The second objective was to evaluate the impact of these treatments on fiber disappearance. Rumen fluid was collected from three lactating cows fed a total mixed diet based on corn silage, soybean meal, linseed meal, barley, wheat, hay and minerals. 200 ml bottles were prepared under anaerobic conditions, with 90 ml of culture medium, 10 ml of rumen juice as inoculum, 2 g DM of substrate and one of the tested PE, or no product for control. The substrate was the diet of donor cows. All bottles were incubated in a water bath stirred at 39°C for 24 hours. Each treatment was tested in a triplicate. Gas production and composition were measured every 6 hours. Fiber disappearance (dFiber) and VFA production were measured after 24h fermentation from a pool of the triplicates. An analysis of variance (Mixed procedure, SAS) was used to evaluate the effect of PE. LS-Means were compared using the Tukey-Kramer method. The significance threshold was set at $p<0.05$. The highest CH_4 yield was measured for the control bottles without PE (Table 1). All the treatments containing PE significantly reduced gas and CH_4 production. Depending on the PE, disappearance of fibers and VFA production were numerically reduced to varying degrees. Calculating the volume of CH_4 produced per unit of fiber lost made the selection of the most promising treatments, i.e. those that did not inhibit fiber digestion and reduced CH_4 yield per g dFiber compared to the control. Among those, the treatment ME_1 50 ppm seemed the most favorable: while dFiber and VFA production were reduced by 18% and 25% respectively, CH_4 yield was decreased by 62%, suggesting a favorable shift in fermentations to mitigate CH4 emissions. This study confirmed the potential of PE to mitigate CH_4 emissions. Based on this approach combining measurements, the effect on fiber fermentation and methanogenesis appeared to vary deeply depending on the PE and the doses used. The treatments reducing CH_4 production more than fiber digestion could be selected for further tests.

Table 1: Fermentation modulation with plant extracts

Treatments	Total Gas_24h (ml)	CH4_24h (ml)	Total VFA (mmol/L)	dFiber (%)	CH4/g digested fiber (ml/g)	SEM
Mustard extract_2, 250 ppm	144.9e	0.0e	23.1	0.0	0.0	0.06
Mustard extract_1, 250 ppm	228.7b	0.0e	79.7	12.9	0.0	2.8
Garlic essential oil, 250 ppm	234.7b	0.0e	73.0	21.8	0.0	17.2
Mustard extract_1, 50 ppm	232.0b	6.8d	69.8	28.3	12.7	
Cinnamaldehyde, 250 ppm	217.9c	7.7d	67.5	21.4	17.7	0.06
Carvacrol, 250 ppm	226.4bc	10.2c	57.4	20.2	25.0	0.06
Tannins, 2000 ppm	202.5d	14.2b	61.3	5.4	33.3	0.85
Control without PE	256.6a	19.3a	85.6	37.5	33.0	0.079
Treatment effect (p-value)	<0.001	<0.001				

Metabolomic Profiling of Dairy Cows During the Transition Period: Associations with Reproductive Outcome

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Improving reproductive efficiency in dairy cows remains a major challenge for the sustainability and profitability of modern dairy farming. The identification of behavioural patterns or physiological alterations associated with fertility loss could enable a more precise management of reproduction at the individual animal level. Aim of the present study is to identify metabolomic parameters that can explain subfertilty in dairy cows and to evaluate metabolic variations across key physiological stages of the transition period, in order to explain fertility variability among cows and support a more targeted reproductive management. The trial was conducted in two medium-sized commercial dairy farms where 137 multiparous Holstein cows were monitored from dry-off until the second artificial insemination (AI). Animals enrolled were managed using two different reproductive strategies: half underwent a Double-Ovsynch synchronization protocol and the other half were inseminated following a precision-based strategy relyng on natural heat detection through AMS. Metabolomic analyses were performed by retrospectively classifying animals according to reproductive outcome. Based on pregnancy diagnosis, cows were divided into Fertile (pregnant within the study period, n=63) and Subfertile (two consecutive negative pregnancy diagnoses, n=74) groups. For metabolomic analyses, blood samples were collected from the coccygeal vein into tubes without anticoagulant at 21 days pre-partum, 10 and 35 days post-partum, at 1°AI and at the eventual 2°AI. Samples were centrifuged at 3000 rpm for 15 minutes to obtain serum, which was stored at -80°C until analysis. Serum metabolomic profiling was performed using a $_1$H-NMR based approach. Low molecular weight metabolites were separated by ultrafiltration (3 kDa cut-off) to remove proteins and macromolecules; spectra were acquired at 600 MHz using a CPMG pulse sequence. After manual phase, spectra were binned and normalized to generate a data matrix for statistical analysis. Multivariate analyses were performed in SIMCA to explore metabolic differences between Fertile and Subfertile groups and across timepoints. Spectral regions contributing most to group separation were assigned to specific metabolites using Chenomx software, and metabolite identification was confirmed by 2D NMR (TOCSY). Based on the results, a targeted analysis of selected metabolites was conducted to improve biological interpretation of the metabolic changes observed. Multivariate models did not show a statistically significant metabolic discrimination between Fertile and Subfertile cows ($p>0.05$). Conversely, a consistent metabolic shift was observed across timepoints, particularly between late gestation (-21) and early lactation (+10, +35). The separation between pre- and post-partum samples reflects the metabolic imbalance that characterizes the transition period in dairy cows. Inter-individual biological variability and spectral complexity likely contributed to the limited separation between reproductive outcome groups. These findings suggest that reproductive failure may not be defined by a single metabolic signature, but future investigations focusing on key metabolic pathways could help differentiate between fertile and subfertile cows. Funded by European Union, Next Generation EU.

Lipidomic profile of dairy cows across key reproductive timepoints and association with fertility outcomes

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Improving reproductive efficiency in dairy cows is essential for the sustainability and profitability of modern dairy farming. Metabolic adaptations occurring around calving and during early lactation may influence reproductive performance and contribute to variability in fertility among animals. Lipidomic profiling provides a useful approach to investigate physiological changes in lipid metabolism associated with reproductive success. The aim of this study was to describe the lipidomic profile of dairy cows across key reproductive timepoints and to evaluate potential differences between cows with different fertility outcomes. The trial was conducted in two medium-sized dairy farms where 137 multiparous Holstein cows were monitored from dry-off until the second artificial insemination (AI). Blood samples for lipidomic analysis were collected at −21 days before calving, 10 and 35 days in milk (DIM), and at first AI. From each blood sample the membranes of the erythrocytes were isolated and by Gas-Chromatography a group of 10 FAs consisting of 2 saturated FA (SFA: stearic, palmitic), 3 mono-unsaturated FA (MUFA: palmitoleic, oleic, vaccenic), 3 polyunsaturated FA ω-6 (PUFA-ω6: linoleic, dihomo-gamma-linolenic [DGLA], arachidonic[ARA]) and 2 PUFA ω-3 (eicosapentaenoic [EPA], docosahexaenoic [DHA]) was determined, along with additional lipid parameters (SFA/MUFA, SFA/PUFA, ω6/ω3, PUFA balance, unsaturation [UI] and peroxidation [PI] indexes). Cows were retrospectively classified according to reproductive outcome as pregnant at first insemination (F1) or not pregnant after two inseminations (NF2). Fatty acid composition and lipid indices were compared between fertility groups at each sampling timepoint. Statistical analyses were performed using RStudio (version 2023.12.1). Overall, most lipid variables were similar between F1 and NF2 cows across the evaluated timepoints. However, a significantly higher concentration of vaccenic acid (18:1 11c) was observed in NF2 cows compared with F1 cows at 10 DIM ($P < 0.05$). Conversely, docosahexaenoic acid (DHA) levels at the time of first AI were significantly higher in F1 cows than in NF2 cows ($P < 0.05$). No other significant differences were detected between fertility groups for the remaining fatty acids or lipid indices at the investigated timepoints. These findings indicate that the overall erythrocyte membrane lipidomic profile was largely comparable between cows with different fertility outcomes. Nevertheless, specific fatty acids, particularly vaccenic acid in early lactation and DHA at the time of insemination, may reflect subtle metabolic differences associated with reproductive success. Further studies are warranted to clarify the potential role of these lipid biomarkers in supporting reproductive efficiency in dairy cows.

Tail hair $\delta^{15}N$ identifies cows with lower calf loss and greater liveweight production in extensive beef systems

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Low protein availability constrains productivity in extensive beef systems. When cattle consume the same diet, the natural abundance of the stable isotope ^{15}N in tail hair ($\delta^{15}N$) reflects nitrogen retention relative to excretion and provides an integrated indicator of nitrogen use efficiency, with lower $\delta^{15}N$ values associated with reduced urinary nitrogen loss. This study evaluated whether $\delta^{15}N$ measured early in the production year could identify cows and heifers with lower calf loss risk and higher liveweight production under protein-limited grazing conditions. Tail hair samples were collected from 4,573 cows and heifers across eight properties in 2022/23 and 12 properties in 2023/24. Tail hair $\delta^{15}N$ was quantified using an IsoPrime100 isotope-ratio mass spectrometer, with values expressed relative to the AIR international standard. Reproductive and productivity outcomes included calf survival to weaning, total reconception, pregnancy within four months post-partum, calf weaning weight, and annual liveweight production. Associations between $\delta^{15}N$ and performance outcomes were analysed using generalised linear mixed models for binary traits and linear mixed models for continuous traits, with $\delta^{15}N$ fitted as a fixed effect and property included as a random effect to account for environmental heterogeneity. Cows with lower $\delta^{15}N$ values had a significantly lower risk of calf loss ($P < 0.01$). Across the observed $\delta^{15}N$ range (approximately 4–12‰), the predicted probability of calf loss declined markedly. Although individual properties spanned narrower $\delta^{15}N$ ranges (typically 2–3‰), the association was consistent across herds. Each 1‰ decrease in $\delta^{15}N$ reduced the odds of calf loss by approximately 23%. Lower $\delta^{15}N$ values were also associated with higher calf weaning weight ($P < 0.01$), with a significant interaction with calf sex ($P = 0.01$). For every 1‰ reduction in $\delta^{15}N$, weaning weight increased by 2.5 kg in male calves and 4.2 kg in female calves. Annual liveweight production (calf weaning weight plus maternal liveweight change) increased by 9.6 kg per cow per 1‰ reduction in $\delta^{15}N$ ($P < 0.01$), and this effect did not differ between sexes. No association was detected between $\delta^{15}N$ and either reconception or pregnancy within four months post-partum ($P > 0.10$). This study provides the first large-scale evidence that $\delta^{15}N$ in tail hair is associated with calf survival and total cow–calf liveweight production in extensive beef systems. Lower $\delta^{15}N$ values likely reflect greater urea recycling to the rumen and reduced urinary nitrogen excretion, enabling increased retention of absorbed nitrogen for maternal maintenance and milk production. These findings position $\delta^{15}N$ as a practical phenotypic indicator of nitrogen efficiency with implications for both productivity and nitrogen-loss mitigation in grazing systems.

Serum microRNA Signatures Associated with Clinical and Subclinical Endometritis in Dairy Cows

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Endometritis remains a major cause of subfertility and economic loss in dairy production, yet reliable biomarkers for early and specific diagnosis are still lacking. Circulating microRNAs (miRNAs) are attractive candidates due to their regulatory roles in inflammation, immunity, and tissue repair, and their stability in serum. This study aimed to identify serum miRNA signatures associated with clinical (CE) and subclinical (SE) endometritis at different postpartum stages, providing both mechanistic and diagnostic insights. Dairy cows were enrolled on the day of calving. At day 28 postpartum (pp), cows underwent a gynecological examination including vaginoscopy. Cows showing pus in the vaginal discharge were diagnosed with CE. Cytobrush samples were collected from cows with clear vaginal discharge to assess the proportion of polymorphonuclear neutrophils (PMN). Animals with ≥5% PMN were classified as having SE, while cows with <5% PMN were considered healthy (HE). In total, 18 cows were included (n = 6 per category: CE, SE, HE) in this study. Individual blood samples were collected longitudinally at 14, 28, and 56 days resulting in 18 serum samples per group (54 total) for global miRNA profiling, followed by Venn analyses to identify shared and unique expression patterns across groups and time points. Key miRNAs were assessed for their relevance to inflammation and epithelial repair. Distinct and time-dependent miRNA patterns were observed. At day 14 pp, 252 miRNAs were shared among all groups, whereas 30, 31, and 37 were unique to CE, SE, and HE, respectively. CE and SE shared 45 miRNAs not detected in HE, indicating early inflammatory overlap. Similar patterns persisted at day 28 pp (232 shared; 64 CE–SE overlap) and day 56 pp (225 shared; 33 CE–SE overlap). Across all time points, 293 miRNAs were consistently detected, representing a stable circulating core. Several miRNAs displayed dynamic, condition-specific profiles. Bta-miR-21-3p shifted from being CE & SE-shared at day 14 pp to SE-unique at day 28 pp and CE-unique at day 56 pp, suggesting a role in inflammatory activation and later tissue remodeling. Bta-miR-141 remained SE-unique across all time points, consistent with involvement in epithelial repair and barrier integrity. Bta-let-7a-3p was shared early but became CE-unique late postpartum, possibly reflecting persistent inflammatory processes. In contrast, bta-miR-146a/b, bta-miR-155, and bta-miR-223 were shared among all groups, indicating general immune regulation rather than disease specificity. Serum miRNA profiling revealed stable and disease-specific expression patterns that reflect the transition from inflammation to recovery in postpartum cows. Early overlap between CE and SE suggests common inflammatory origins, while later divergence indicates distinct regulatory pathways. Among the identified miRNAs, miR-21-3p and miR-141 emerge as promising biomarkers combining diagnostic potential with mechanistic relevance, supporting their inclusion in multi-miRNA panels for early, stage-specific detection of endometritis.

Selection for Developmental Speed Skews Sex Ratios and Molecular Profiles in Bovine IVP Embryos

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The developmental pace of in vitro produced preimplantation embryos is widely used to select candidates for transfer, yet the molecular profiles captured by fast versus slow development and the potential impact of this selection on embryo physiology and sex ratio remain largely unclear. Here, we investigated whether bovine IVP blastocysts that hatch on Day 7 versus Day 8 differ in transcriptomic and metabolic features, and whether this selection window alters the sex ratio. Hatched blastocysts collected on Day 7 and Day 8 were profiled by RNA sequencing and complemented with measurements of lipid accumulation, mitochondrial activity and reactive oxygen species (ROS). Embryo sex was determined by Y-chromosome multiplex PCR, and selected transcripts were validated by qPCR in individually sexed embryos. RNA-seq revealed 256 differentially expressed genes (DEGs) between Day 7 and Day 8 hatched blastocysts. Among these, 79 transcripts showed higher and 177 showed lower abundance in fast developing embryos (Day 7) relative to slower developing embryos (Day 8). Notably, Day 8 blastocysts showed higher expression of so-called pregnancy recognition factors (IFNT2, IFNT3) and the trophoblast marker TKDP1, as well as genes associated with glycolysis, lipid transport, and endoplasmic reticulum stress. In contrast, Day 7 blastocysts displayed upregulation of a number of genes involved in oxidative phosphorylation. Consistently, Ingenuity Pathway Analysis identified Oxidative Phosphorylation, Estrogen Receptor Signaling, and IL-12 Signaling as highly upregulated canonical pathways in fast-developing embryos, whereas Unfolded Protein Response, Glycolysis I, Endoplasmic Reticulum Stress, and Sirtuin Signaling were upregulated in Day 8 embryos. Despite these transcriptomic differences, mitochondrial membrane potential and ROS levels were not significantly different between groups, whereas Day 7 hatched embryos contained significantly greater neutral lipid accumulation. Sexing analysis demonstrated a male-biased sex ratio among hatched embryos on Day 7 (66.7%) and Day 8 (58.5%), whereas embryos hatching on Day 9 did not show a significant male skew. qPCR validation identified sex dependent regulation of UQCRC2 and hatching day dependent regulation of ENO1, UQCRC2, HSP90B1, and APOA1, highlighting interactions between sex and developmental timing in pathways linked to energy metabolism and stress responses. Together, these data show that Day 7 and Day 8 hatched bovine IVP blastocysts represent separable molecular and metabolic states. Importantly, embryo selection based on rapid development can inadvertently shift both the sex ratio and the underlying molecular profile of transferred embryos, with potential consequences for pregnancy establishment and herd replacement strategies.

Extender-Specific Lipidomic Signatures in Buck Semen

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Semen extenders play a central role in artificial insemination and sperm cryopreservation by providing structural protection and metabolic support to sperm cells. Sperm lipid composition is a critical determinant of membrane functionality, influencing capacitation, acrosomal stability, resistance to oxidative stress, and cryosurvival. The objective of this study was to compare the lipidomic profiles of different semen extenders used in buck semen cryopreservation. Sperm samples (n=10, bucks) were diluted with plant (AndroMed® and Bovifree) and animal (Oviplus, Steridyl, and Triadyl) based extenders. Following a 4h equilibration at +4 °C, samples with total motility above 70% were cryopreserved, cooled from +5 °C to liquid nitrogen vapours -100 °C for 15 min, and stored in liquid nitrogen until post-thaw evaluation. Sperm samples containing 200 × 106 sperm/mL were washed by centrifugation at 2500× g at 4 °C for 10 min. Then, supernatant (extender and seminal plasma) was discarded. Lipids were extracted using a chloroform–methanol (2:1) solution and dried lipids were stored at −20 °C until further analysis. Gas chromatography–mass spectrometry analysis was conducted using an Agilent 7890A GC System coupled to an Agilent 5975C inert XL MSD with the triple-axis mass detector. Principal Component Analysis (PCA) revealed a clear separation among semen extenders based on their lipid composition. The first principal component (PC1) accounted for 90.3% of the total variance, while the second principal component (PC2) explained 4.6%. Along PC1, plant-based extenders clustered together on the negative axis, whereas animal-based extenders clustered on the positive axis. We identified a total of 33 fatty acids across all semen extenders. Among these, eleven fatty acids, C14:1, C14:0, C15:0, C16:1, C16:0, C17:0, C18:2, C18:1 (cis-9), C18:0, C20:4, and C22:6 showed significant differences among the extenders ($p < 0.05$). In contrast, C22:0 did not differ significantly between extenders ($p > 0.05$). The findings indicate that semen extenders with different formulations are associated with distinct fatty acid profiles and differences may be considered when selecting extender formulations for semen processing and cryopreservation. Keywords: Sperm, buck, lipidomics, fatty acid, cryopreservation

Reproductive performance as a pillar of pig production: interaction between parity and barn environmental parameters

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Seasonal infertility compromises reproductive efficiency in pig production by increasing biological losses and reducing the sustainability and profitability of livestock systems. Developing more adaptive production systems and sows requires understanding how environmental factors interact with reproductive performance under different climatic conditions. This study evaluated the associations between in-barn heat load (HL) and light variability (L) with the reproductive outcomes farrowing success (FS) and litter size (LS) in purebred Large White sows raised in Brazil under contrasting subtropical and tropical production systems. We hypothesized that higher HL and greater light variability could affect FS and LS, with effects differing according to farm location and climate. Reproductive records from 2021 were retrospectively analyzed from two commercial Brazilian farms: a subtropical system in the southern region (Farm 1) and a tropical system in the central-west region (Farm 2). Temperature (°C) and photoperiod (lx) in gestation facilities were monitored using data loggers. Heat load was calculated using a plateau-linear function considering 25 °C as the upper critical temperature, while light variability was quantified as deviations from the expected daylight illuminance profile. Associations with FS and LS were evaluated using logistic and linear regression models, respectively. Results were expressed as odds ratios (OR) for FS and regression coefficients (β) for LS, with statistical significance ($P < 0.05$). Overall, environmental effects on reproductive performance were modest but varied according to production context. In the combined analysis of both farms, HL was not associated with FS ($P > 0.05$) but showed a modest positive association with LS ($\beta = 0.281$; $P = 0.031$). Light variability was not associated with either FS ($P = 0.836$) or LS ($P = 0.728$). A farm effect was observed for FS, with higher odds of success in Farm 2 compared with Farm 1 (OR = 1.724; $P = 0.029$), suggesting the influence of farm-specific factors such as housing design, environmental control, or management practices. In the farm-specific analyses, HL was not associated with FS or LS in Farm 1 (subtropical system) ($P > 0.05$). However, light variability was positively associated with LS ($\beta = 0.355$; $P = 0.014$), without affecting FS. In contrast, in Farm 2 (tropical system), light variability was negatively associated with both FS (OR = 0.72; $P = 0.028$) and LS ($\beta = -0.59$; $P = 0.020$), while HL remained unrelated to the outcomes. These results suggest that instability in light conditions may represent a relevant challenge for reproductive stability in this production context. In summary, the findings indicate that the reproductive impact of in-barn environmental conditions is system-dependent rather than uniform across climatic contexts. While heat load showed limited associations with reproductive outcomes, light variability emerged as a relevant factor, particularly under tropical conditions. The differences observed between farms highlight that environmental stressors operate within the context of baseline climate and production structure, emphasizing the importance of farm-specific environmental monitoring and control strategies to improve reproductive efficiency.

Effect of dietary quercetin and genetic line in rabbit semi-intensive production system

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Rabbit production is a sustainable livestock system due to its low environmental impact. The semi-intensive production system overlaps gestation and lactation, leading to negative energy balance and increased oxidative stress, which impairs reproductive performance and shortens lifespan, affecting productivity. Management strategies that improve circularity such as antioxidant supplementation with quercetin (Q) and the use of resilient genetic lines (GL) may help to optimise reproductive and productive efficiency. Does of two GL: V line (selected for litter size at weaning; group V) and LP line (selected for productive longevity, group LP) were fed a supplemented diet with 4g/kg Q (group Q) or not (group C) during rearing and the first four reproductive cycles under a semi-intensive system. Effect of Q and GL on body composition (BC) (fat and energy content), fertility, prolificacy and milk yield (MY) along cycles was evaluated. Results showed that Q did not affect BC, fertility, prolificacy or MY. LP line showed lower body fat and energy reserves compared with V line (fat:11.93±0.3 vs 10.94 ± 0.3%; p=0.03, and energy:982.18±13.9 vs 941.40±13.7kJ/100g; p=0.04, respectively), but higher MY (LP:5.6±0.1 vs V:5.2±0.1kg, p<0.01). LP does were able to maintain fertility in cycle 2 (55.5±7.5%). Similar number of liveborn kits was found between lines (LP:10.0±0.5 and V:10.6±0.5). The lowest BC was reached at first weaning (fat:7.6±0.4% and energy:769.46±21.4kJ/100g, p<0.0001), and the fertility decreased at second parturition (48.3±5.4%, p<0.001). Prolificacy and MY increased with the number of reproductive cycles (p<0.0001). In conclusion, Q had limited effects on the parameters studied. However, LP line demonstrated its functional robustness with better mobilization of reserves and higher milk production. Parity order affected reproductive performance, being particularly challenging for primiparous does, which has higher energetic demands. This work was supported by Grant PID2022-138223OB-I00 (funded by MCIN /AEI /10.13039/501100011033 [Spain], FSE, FEDER [EU] and, Next Generation EU/PTR)

Assessment of Securidaca longipedunculata (violet tree) effect on Semen Quality and Blood Sex Hormone Levels in Indigenous Goats

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Assessment of Securidaca longipedunculata (violet tree) effect on Semen Quality and Blood Sex Hormone Levels in Indigenous Goats Kamogelo. Shai, Segolo L. Lebelo, Freddy. Manyeula, Monnye. Mabelebele, Amenda N. Sebola a Department of Agriculture and Animal Health, College of Agriculture and Environmental Sciences, University of South Africa, Florida, 1710, South Africa bDepartment of Life and Consumer Sciences, College of Agriculture and Environmental Sciences, University of South Africa, Florida, 1710, South Africa Presenting author Dr K Shai: E-mail address. shaik@unisa.ac.za Abstract Semen quality and endocrine function are critical determinants of reproductive efficiency in indigenous goat bucks (IGB). Securidaca longepedunculata contains bioactive phytochemicals such as flavonoids, saponins, and alkaloids with antioxidant and potential androgen-modulating properties. It was hypothesised that supplementation with S. longepedunculata leaf extract (SLLE) would enhance semen quality and influence reproductive hormone concentrations in IGB. Sixteen (16) pubertal bucks (12–18 months) were randomly allocated to four treatment groups (n = 4 per treatment) receiving 0, 50, 100, and 125 mg/kg body weight of SLLE. Semen characteristics and serum hormones were analysed using a completely randomized design in SAS (PROC GLM). Data were pooled across time and subjected to one-way ANOVA, with mean separation using PDIFF at $P < 0.05$. SLLE significantly affected semen characteristics. Semen volume increased ($P = 0.01$) from 0.26 mL (control) to 0.42 and 0.38 mL at 50 and 100 mg/kg, respectively, but declined to 0.15 mL at 125 mg/kg. Sperm concentration improved ($P = 0.001$) from 267.3 $\times 10^6$/mL (control) to 441.5 and 455.6 $\times 10^6$/mL at 50 and 100 mg/kg, respectively, before decreasing to 169.3 $\times 10^6$/mL at 125 mg/kg. Total sperm motility increased ($P = 0.015$) from 29.0% (control) to 67.8% and 63.5% at 50 and 100 mg/kg, respectively. Similarly, progressive motility improved ($P = 0.02$) from 17.4% (control) to 49.8% and 45.4%, while rapid motility increased ($P = 0.02$) from 15.4% to 45.3% and 40.0% at 50 and 100 mg/kg, respectively. Sperm membrane integrity was higher ($P = 0.015$) at 50 (63.8%) and 100 mg/kg (60.9%) compared to the control (39.2%) and 125 mg/kg (36.3%). Live sperm percentage increased ($P = 0.005$) from 35.8% (control) to 78.8% at 100 mg/kg and 79.0% at 125 mg/kg. In contrast, serum testosterone (6.63 vs. 5.1–5.9 ng/mL) and oestrogen (0.83 vs. 0.60–0.88 ng/mL) were not affected ($P > 0.05$) by SLLE supplementation. In conclusion, moderate SLLE supplementation (50–100 mg/kg) significantly improves semen quality and sperm motility parameters in IGB, likely through antioxidant-mediated mechanisms, while higher doses may exert detrimental effects on certain traits. The lack of hormonal response suggests that improvements in semen quality are not mediated through endocrine changes. However, the small sample size (n = 4 per treatment) may limit statistical power for highly variable reproductive traits, and further studies with larger cohorts are recommended. Keywords: Securidaca longepedunculata, semen quality, sperm motility, indigenous goats, phytochemicals, reproductive hormones

Effects of maternal undernutrition on splenic white pulp morphology and immune-related metabolomic profiles in Japanese Black (Wagyu) fetuses

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Fetal underdevelopment increases the risk of neonatal disease and may adversely affect subsequent growth and fattening performance. Therefore, appropriate development of immune organs during early life is essential. The spleen is a secondary lymphoid organ and plays a key role in immune responses. Maternal nutrition is known to influence fetal development, and maternal undernutrition has been shown to reduce fetal body, thymus, and spleen weights. Splenic transcriptome analyses have further suggested altered inflammatory signaling and compensatory hematopoiesis under maternal undernutrition. In the present study, we evaluated splenic white pulp morphology and performed metabolomic profiling to clarify the effects of maternal undernutrition on the fetal spleen. Twelve multiparous Japanese Black cows were assigned from conception to day 260 of gestation to either a control group (CON, n = 6) receiving 120% of the Japanese Feeding Standard or a maternal undernutrition group (MUN, n = 6) receiving 60%. At day 260, fetuses were harvested and fetal spleens were collected. Hematoxylin and eosin staining was performed, and the white pulp area was quantified. Splenic metabolites were analyzed using LC-TOFMS. Differential metabolites were identified using Welch's t-test ($p < 0.1$), followed by pathway enrichment analysis. Maternal undernutrition significantly reduced white pulp area in the fetal spleen ($p < 0.01$). Metabolomic analysis detected 338 metabolites and identified 28 altered metabolites ($p < 0.1$). Notably, taurine, adenine, cytidine, 2′-deoxycytidine, 5-methylcytidine, GMP, phosphorylcholine, CDP-choline, dopamine-related compounds, and serotonin were reduced in the MUN group. Pathway analysis indicated enrichment of broad metabolic pathways and neuroactive ligand–receptor interaction pathway. Many altered metabolites are involved in nucleotide and choline metabolism, essential for cell proliferation and membrane synthesis. Their coordinated reduction, together with decreased white pulp area, suggests limited substrate availability for lymphoid cell development under maternal undernutrition. Reduced monoamine-related metabolites may reflect alterations in the splenic neuroimmune microenvironment. In conclusion, maternal undernutrition induces morphological and metabolic alterations in the fetal spleen, characterized by reduced white pulp development and metabolic downregulation. These findings indicate that maternal nutritional status influences immune organ development during gestation and may affect postnatal immune function.

Computer-Assisted Evaluation of Different Diluents on Kinematic Parameters of Ram Semen During Liquid Storage up to 72 h at 4 °C and after 2 h of Incubation

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The preservation of ram semen quality during liquid storage is strongly influenced by diluent composition and sperm resistance to thermal stress after warming. This study evaluated the effects of different diluent formulations on ram semen quality during storage and after a 2 h incubation at 38 °C. Semen pools from six rams were diluted using eight extender formulations containing combinations of dimethylformamide (DMF) at 1.5%, Tempol (T) at 0.34 mg/mL, and hyaluronic acid (HA) at 0.06 mg/mL, with OviXcell® as reference. Samples were stored in liquid form at 4 °C and evaluated by CASA at 0, 24, 48 and 72 h after dilution. At each time, sperm quality was assessed immediately after warming to 38 °C and again after an additional 2 h incubation. Parameters included total motility, progressive motility and kinematic parameters included curvilinear velocity (VCL), straight-line velocity (VSL), average path velocity (VAP), linearity (LIN), straightness (STR), and amplitude of lateral head displacement (ALH). Data were analyzed using linear mixed-effects models with diluent, storage time and incubation as fixed effects and semen pool as a random effect. Storage time and incubation significantly affected all semen quality parameters. Total motility was influenced by storage time ($F = 6.55$, $p = 0.0004$) and incubation ($F = 24.10$, $p < 0.001$), while progressive motility showed strong effects of storage ($F = 8.21$, $p < 0.001$) and incubation ($F = 76.49$, $p < 0.001$). Significant storage × incubation interactions were observed for total motility ($p = 0.025$) and progressive motility ($p = 0.010$), indicating that incubation effects increased with storage duration. Velocity parameters were strongly affected by both factors. Motile VCL, VSL and VAP were significantly influenced by storage (VCL: $F = 59.19$; VSL: $F = 155.92$; VAP: $F = 172.40$; all $p < 0.001$) and incubation (VCL: $F = 82.45$; VSL: $F = 343.29$; VAP: $F = 139.53$; all $p < 0.001$). Significant storage × incubation interactions ($p \leq 0.005$) showed that velocity declined more markedly after incubation at longer storage times. Diluent had limited effects on total motility but significantly affected several kinematic parameters, including motile VSL ($F = 3.33$, $p = 0.003$), motile LIN ($F = 4.69$, $p < 0.001$), motile STR ($F = 2.29$, $p = 0.031$), progressive VSL ($F = 2.48$, $p = 0.020$), progressive LIN ($F = 5.89$, $p < 0.001$) and progressive STR ($F = 2.09$, $p = 0.049$). Significant diluent × storage interactions were detected for total motility ($p = 0.028$), progressive motility ($p = 0.020$), motile LIN ($p = 0.013$) and progressive LIN ($p = 0.006$). Diluent × incubation interactions were also observed for motile VCL ($p = 0.018$), motile LIN ($p < 0.001$), motile STR ($p = 0.038$), motile ALH ($p = 0.040$), progressive VCL ($p = 0.019$) and progressive LIN ($p < 0.001$), indicating differences in sperm resistance to thermal stress. Pairwise comparisons showed that 1.5 DMF + T + HA maintained among the highest progressive motility and kinematic values after prolonged storage and incubation. Storage duration and incubation at physiological temperature strongly affect ram semen quality, while diluent composition modulates sperm resistance to incubation stress. Extenders combining DMF with Tempol and HA improved preservation of sperm motility and kinematic characteristics during storage and subsequent incubation.

Effects of egg yolk and goat milk extenders on cryopreserved semen quality of West African Dwarf goats

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Abstract Egg yolk and goat milk are widely used as cryoprotective extenders to enhance sperm motility, plasma membrane functionality, acrosome integrity and fertilizing ability in farm animals. However, their comparative effectiveness in preserving semen quality of West African Dwarf (WAD) goat bucks during cryopreservation has not been well established. This study evaluated the effects of Tris-based egg yolk and goat milk extenders on motility and functional integrity of WAD goat spermatozoa incubated at different temperatures. Pooled semen samples were collected from two mature bucks across five ejaculates (n = 5 replicates) and diluted in Tris egg yolk or Tris goat milk extender. Diluted semen samples were divided into five aliquots and incubated at 20, 16, 12, 8 and 4 °C. Samples were gradually cooled from 20 °C to 4 °C at a rate of 0.25 °C/min, equilibrated, and subsequently cryopreserved in liquid nitrogen (−196 °C) for 24 hours. Following thawing, sperm motility, acrosome integrity, membrane integrity, livability and morphological abnormalities were evaluated. Data were analysed using analysis of variance in a 5 × 2 factorial arrangement within a completely randomized design, and means were separated using Duncan's Multiple Range Test at $P < 0.05$. Results showed that higher sperm motility was obtained at 4 °C in the Tris egg yolk extender and at 16 °C in the Tris goat milk extender compared with other incubation temperatures. Membrane integrity was comparable between both extenders. Egg yolk extender maintained higher motility at lower temperature (4 °C), while the highest acrosome integrity was observed in semen extended with Tris egg yolk and incubated at 16 °C. These findings demonstrate that both extender type and incubation temperature significantly influence cryosurvival of WAD goat spermatozoa, with egg yolk extender showing superior performance in maintaining sperm motility at lower temperatures. Keywords: Cryopreservation; cryoprotectants; sperm motility; cooling temperature; extenders; West African Dwarf goat.

Policy to practice: Driving farm-level change with carbon accounting and transparent Scope 3

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The need for accurate, cost-effective carbon accounting in agriculture has intensified as policy and markets demand robust measurement, reporting and verification (MRV) of both emissions and removals. Tier 1 factors have proven insufficient for capturing farm-level realities or tracking genuine progress towards net zero. This has driven efforts to develop Tier 2 (and eventually Tier 3) factors, particularly for carbon removals in productive landscapes where complexity arises from diverse soils, management practices and livestock systems. John Gilliland, a practitioner and farmer, with long-standing involvement in UK and international agricultural climate policy has contributed to this challenge through progressively scaled projects that combine accredited farm carbon calculators (initially with Tier 1 removals modules) with site-specific measurement techniques. The ARC Zero project (EIP-Operational Group, Northern Ireland, 2020–2023) serves as a concrete early example. Working with seven diverse farms (~930 ha, covering varied soil types and enterprises), the project established whole-farm baselines using Agrecalc. It integrated high-resolution aerial LiDAR surveys (40 scans per m²) for above-ground biomass quantification and deeper soil organic carbon sampling (to 1 m) to better capture contributions from livestock manure, multi-species swards and complex root systems. Preliminary results showed that 97% of total carbon stocks resided in the soil rather than in trees or above-ground biomass. Across the seven farms, combined carbon stocks totalled 515,166 t CO_2e, with soil carbon accounting for 494,438 t CO_2e (top 30 cm, conservative bulk density). Two farms were already at net zero status (gross emissions minus sequestration) at the outset. On the five farms with follow-up data, emission intensity reductions ranged from 4% to 53% through changes such as reduced nitrogen fertiliser, clover introduction and multi-species swards. These outcomes highlighted farms as significant carbon custodians while exposing limitations of standardised Tier 1 approaches in heterogeneous landscapes. This work has scaled to the AHDB-led Environment Baselining Pilot (supported by Quality Meat Scotland), now in its second year of a five-year programme involving 178 farms and over 36,000 ha across Great Britain. Initial baselines are complete, with early observations indicating that 14% of participating farms (more than half with livestock) are already beyond net zero on a net carbon basis. Soil carbon again comprises around 95% of measured stocks. Full peer-reviewed publication of results from this larger programme is still pending. John now advises the EU-funded CAFAMORE project, developing a cost-effective hybrid MRV solution (Earth Observation, Tier 3 modelling and ground truthing) for the EU Carbon Removals Certification Framework, and contributes to the UK Carbon Removals Taskforce on the GHG Protocol's Land Sector Removals Standards for Scope 3 declarations. These projects illustrate the tension between standardised, scalable carbon accounting and the need to reflect site-specific biophysical realities. Early findings emphasise the dominance of soil carbon, the value of hybrid methods (LiDAR + deep soil sampling), and the potential for data-driven management to deliver mitigation alongside co-benefits such as improved soil health. They also underscore the importance of transparent, cost-effective MRV for credible carbon removals claims in food-producing landscapes.

Carbon footprint as a key indicator of structural diversity in extensive dehesa livestock farms

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Extensive livestock farming systems are typically analysed from different perspectives, such as breed type, economic performance and environmental impact. This fragmented approach makes it difficult to gain a full understanding of the diversity of these systems, particularly in a context of increasing climate uncertainty. A more integrated assessment is required in order to better characterise how these farms function. This study evaluated 52 dehesa farms using technical, economic and environmental indicators during 2021–2022. Principal Component Analysis was followed by Cluster Analysis to identify the main structural characteristics. Three key factors were identified: (i) the level of intensification and greenhouse gas emissions; (ii) land tenure and labour; and (iii) dependence on Common Agricultural Policy (CAP) subsidies. These three factors explained 67.63% of the total variance, enabling the classification of four farm groups. The results showed that farms with lower levels of intensification (clusters 1, 2 and 3) had a lower environmental impact (991.99, 727.20 and 1,049.87 kg CO2eq per hectare per year, respectively) and were less reliant on external inputs. In contrast, the most intensive cluster (cluster 4) had an environmental impact of 2,183.58 kg CO2eq per hectare per year, though it achieved better economic performance. Of the identified groups, cluster 3 was the most balanced, combining good technical and economic results with regenerative management practices. This typology highlights the importance of incorporating greenhouse gas emissions into analyses of extensive livestock farms. It provides a basis for developing management strategies aimed at enhancing sustainability and resilience under climate uncertainty.

A combined nutritional-genetic modeling approach to predict enteric methane emissions in individual and groups of dairy cows

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Both nutrition and genetic selection for lower enteric methane (CH4) emissions can deliver permanent and cumulative strategies to lower CH4 production from dairy cattle. In 2023, Canada released CH4 breeding values derived from mid-infrared spectral data of milk samples and rigorous CH4 measurements collected using GreenFeed (C-Lock Inc., USA) in Holstein cows. Previous studies reported moderate heritability for daily CH4 production, CH4 yield, and CH4 intensity (ranging from 0.16 to 0.40), with a strong genetic correlation between CH4 production and intensity (rg = 0.94), supporting the inclusion of a CH4 trait in selection programs. Concurrently, numerous animal trials have demonstrated that nutritional manipulation can reduce enteric CH4 emissions in dairy cows, and both empirical and mechanistic models have been developed to predict CH4 emission responses to these dietary strategies. To quantify reductions achieved by combined mitigation measures, integration of genetic and nutritional mitigation strategies into carbon accounting tools are required. Currently, no model integrates both methane estimated breeding values (EBVs) and predicted CH4 emissions from nutritional models for concurrent use in dairy cows. Therefore, this study aimed to develop models that combine empirical prediction of CH4 based on nutritional variables with CH4 breeding values to predict enteric CH4emissions from individual dairy cows. Observed data was provided by the consolidated database of Net-zero Dairy Genome Project (www.ndgp.ca) collected from the Ontario Dairy Research Centre, Elora, ON, Canada (ODRC). The dataset included 627 genotyped first parity Holstein cows encompassing 2,974 records for CH4 production, DMI and dietary nutrient composition. Individual CH4 measurements were collected mid-lactation (day 120-185 DIM) using a GreenFeed system. Cows were moved in groups of 2 to 4 into a tie-stall area and acclimatized for 2-days, followed by a 5-day CH4 measurement period (3 to 4 visits a day of 8-10 min each). During this period, cows were fed a total mixed ration ad libitum and had free access to water. Twenty-five published empirical CH4 prediction models were identified and their predictive performance evaluated using the reported CH4, DMI and nutrient composition records from the ODRC herd. Models incorporating DMI, NDF, and fatty acid content of the diet outperformed equations that used only DMI or omitted fibre and fat content of the diet. The top eight performing models, based on the Root Mean Square Prediction Error (RMSPE) and concordance correlation coefficient (CCC), were then combined with CH4 EBVs from selected genotyped herds. Integrating EBVs with the empirical models moderately improved CH4 emission prediction accuracy, reflected by lower RMSPE and higher CCC. These results demonstrate the potential of combining genetic and nutritional models to predict individual CH4 emissions, and supports application in national inventories and life cycle assessment analysis.

Bridging the Climate-Corporate Gap: Utilizing GWP* with GWP100 for Livestock Companies' Greenhouse Gas Inventories

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The global warming potential (GWP) metric on a 100 year basis (GWP100) is currently the most common metric implemented by corporations in their greenhouse gas (GHG) inventories. However, GWP100 cannot adequately account for the short atmospheric lifespan of some GHG, such as CH4 which has a 12-yr atmospheric lifespan. As such, GWP100 is unable to adequately translate GHG emissions to climate warming contributions. More recently, GWP-star (GWP*) has been developed to resolve this issue. The GWP* and GWP100 result in different estimates of climate warming contributions by CH4. With the emergence of GWP* there has been increasing interest in adopting this metric into corporate reporting. However, the 20-year baseline data required to calculate GWP* has limited companies' ability to incorporate GWP* into their current GHG reporting inventories. To bridge the gap between GWP* and corporate accounting we developed a straightforward methodology to enable companies to dual report both GWP* and GWP100 within their inventories. Specifically, the methodology developed a "shadow company" technique that will allow GWP* to be This is a provisional file, not the final typeset article calculated from a specified baseline reporting year and onwards. To realistically model a corporate inventory we developed a mock beef company, titled the Global Beef Company that operated in Brazil, Australia, and the United States. Emission reduction and growth scenarios were modeled over an 80-year timescale. To evaluate the cumulative effect of warming over time, GHG were modeled using cumulative sum of GWP* and GWP100 values. With many companies reporting GHG on an intensity basis, a new metric was formulated entitled Intensity Cumulative where GWP* and GWP100 were both reported on intensity basis. Overall, the paper provides a step-by-step template for dual reporting GWP* and GWP100 on an absolute and intensity basis for GHG corporate accounting.

Dynamic Life Cycle Assessment of Greenhouse Gas Emissions of Live Animals in Beef Cattle Production

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The major sources of greenhouse gas (GHG) emissions in ruminant livestock systems are enteric methane and excreta methane and nitrous oxide emissions. Since emission mitigation potential of these emissions is realised at farm through decisions made at herd and individual animal levels, dynamic tools are needed to quantify emissions and to identify high-emitting animals at these operational scales. Although existing Life Cycle Assessment (LCA) frameworks, such as the Product Environmental Footprint Category Rules (PEFCR) for Dairy Products, the IDF global Carbon Footprint standard for the dairy sector, and the Finnish Food-LCA guidelines, provide general methodologies for quantifying livestock emissions, they are mainly designed for dairy systems and farm- or product-level assessments. Consequently, they require systematic adaptation before they can be applied to animal-level calculations in beef cattle during the growing period. This gap limits stakeholders in developing and evaluating effective mitigation and management strategies. The aim of this work is to develop a dynamic approach for estimating GHG emissions of live animals during the fattening stage at farm. Following the principles laid down in PEFCR Dairy for climate change impact assessment, we developed an animal-level emission calculation model for Finnish beef production systems. The model integrates IPCC Tier 2/3 equations for enteric methane and manure methane emissions, Tier 1/2 approaches for direct and indirect nitrous oxide emissions, and upstream LCA data for feed. Primary data includes daily diet composition, feed composition, live weight, and manure management system. Secondary data is used for emissions from feed production. Capital goods and housing energy are excluded. The model outputs the total GHG emissions from enteric fermentation and manure management per animal per day and per kilogram of live weight, allowing comparability with farm and product level indicators. Application of the model in Finnish beef herds reveals variability in methane intensity, driven mainly by differences in growth rate, diet composition and manure management conditions. These results show that aggregated farm-level metrics can mask significant individual variation and highlight the potential for targeted interventions. The developed model provides stakeholders such as producers, advisors, processors, breeding companies, environmental agencies and researchers with a practical, new tool for evaluating mitigation options, optimising feeding strategies, benchmarking animals, and reporting climate performance using traceable and reproducible calculations. In conclusion, this work demonstrates that existing LCA standards can be systematically adapted to dynamic animal-level assessments of live beef cattle without compromising methodological consistency. The resulting framework contributes a novel approach to integrating individualized GHG emission data into management, breeding and sustainability reporting, supporting the transition toward more climate smart beef production in Europe. Authors received financial support from the European Union's Horizon Europe Coordination and Support Action under grant agreement no. 101134866 (project Digi4Live, https://digi4live.eu/).

Dietary carbon use efficiency, emissions, and losses in growing-finishing pigs

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Carbon (C) accounting is a process of quantifying direct and indirect carbon use, losses, and emissions of agriculture and food supply chains, and is typically measured in carbon dioxide equivalents (CO2e). It is a framework to measure, track, and report emissions to identify hotspots, set reduction targets, meet sustainability goals, reduce operational costs, and ensure regulatory compliance. Livestock and poultry production contributes about 12% to the total global anthropogenic emissions of CO2, CH4, and N2O. Although pigs emit less CO2 and CH4 than ruminants, accurate assessment of the C mass balance and emissions in swine production systems is needed for developing of feeding strategies that improve C use efficiency of pork production to minimize C losses to the environment, spare unnecessary use of natural resources, enhance food security, and reduce the cost of production. Previous estimates of the C footprint of pork production chains are highly variable and range from 0.6 to 6.75 kg CO2e per kg live weight and 2.25 to 4.52 kg CO2e per kg pork products. As a result, the current review is the first to systematically summarize, synthesize, and evaluate empirical published and unpublished data to quantify C mass balance, emissions, and losses in growing-finishing pigs. Thermal combustion analysis is the most common standardized method for determining total C concentrations in a wide variety of matrices including feed ingredients. Analytical results of the C concentration of 80 feed ingredients were highly variable (0.02 to 77% C) within and among classification categories which included: crystalline amino acids (30 to 64% C); animal by-products (38 to 55% C); fats and oils (~ 77% C); inorganic macro- and microminerals (0.02 to 12% C); grains and plant-based by-products (44 to 60% C); and organic trace minerals and choline chloride (1 to 34% C). There was a strong positive association between fecal ($r = 0.998$; $P < 0.001$) and urinary ($r = 0.998$; $P < 0.001$) C and gross energy (GE) excretion, where daily fecal C excretion corresponded to a loss of 10.55 kcal GE per g of C, and urinary C excretion corresponded to a daily loss of 11.91 kcal GE per g of C. Overall, about 13% of dietary C intake is excreted in feces, 3% is excreted in urine, while only about 25% of dietary C intake is retained in whole body protein and fat. Depending on the methods and assumptions used, exhaled C represents the greatest C loss and represents about 55% to 61% of dietary C intake. On a GE basis, 25% of dietary net energy C is retained (10% in body protein, 15% in body fat), 30% of energy is lost from fasting heat production, 29% is lost from heat production from activity and thermic effect of feed, and 13% and 3% of GE is lost in feces and urine, respectively. These results indicate that dietary C use efficiency in growing-finishing pigs is relatively poor (~25%) with more than 55% of dietary C lost from enteric C emissions. Studies are needed to quantify C utilization associated with the physiological and metabolic changes caused by heat stress and immune challenges in pigs. In addition to diets and feeding programs, manure management is the other major contributor to CO2, CH4, and N2O emissions from swine production systems. Type and time period of manure storage affects variability in CO2, CH4, and N2O emissions under commercial conditions which need to be quantified and better characterized.

Economic and greenhouse gas emissions assessment of grass silage under different management scenarios in Irish grassland systems

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Feed provision is the principal variable cost and a key source of greenhouse gas emissions (GHGE) on Irish grassland farms. Grass silage is the predominant conserved forage in Irish livestock systems and management choices such as cutting frequency, conservation method and fertilisation strategy affect both costs and emissions. The Grange Feed Cost Model (GFCM), developed by Teagasc, is a simulation model which integrates agronomic outputs and resource use to calculate total feed cost from crop establishment to feeding; however, it does not include environmental indicators. The objective of this study was to augment the existing model with a GHGE module enabling scenario-based assessment. The economic module accounts for variable and fixed costs and reports results per hectare (ha), per tonne (t) of dry matter fed (DM) and per GJ of metabolisable energy fed (ME). To implement the GHGE module, ISO 14040/44 principles were followed using an inventory-based cradle-to-feeding boundary. The global warming potential (GWP) was calculated using IPCC (AR6) 100-year characterisation factors for carbon dioxide (CO_2), methane and nitrous oxide. Emission factors for upstream inputs were derived from established Life Cycle Inventory databases (Ecoinvent and Agri-footprint) or the literature. Forage production scenarios included perennial ryegrass only (PRG), harvested once or twice, and PRG plus red clover (RC) harvested twice or three times, annually. In terms of management, two conservation methods (pit or baled silage) and two fertilisation strategies (inorganic fertiliser with slurry (WS) or without slurry (WO)) were evaluated. Under the one-cut PRG pit scenario, GHGE were 1.53 t CO_2-eq/ha (31.6 g CO_2-eq/MJ ME) for WS and 1.55 t CO_2-eq/ha (31.9 g CO_2-eq/MJ ME) for WO. Total costs were 307 €/t DM (28.9 €/GJ ME) and 325 €/t DM (30.6 €/GJ ME), respectively. In the two-cut PRG pit scenario, GHGE increased to 2.59 t CO_2-eq/ha (32.4 g CO_2-eq/MJ ME) under WS and 2.70 t CO_2-eq/ha (33.8 g CO_2-eq/MJ ME) under WO, with total costs of 339 €/t DM (32.2 €/GJ ME) and 354 €/t DM (33.6 €/GJ ME), respectively. Baled silage increased GHGE and cost per ha by approximately 7% but reduced GHGE and cost per MJ of ME fed by 3% compared with pit conservation. Across PRG scenarios, slurry use reduced GHGE by 3% and total costs by 4%. RC swards exhibited markedly lower GHGE. Two-cut RC pit silage produced 1.36 t CO_2-eq/ha (16.2 g CO_2-eq/MJ ME fed), while baled silage produced 1.42 t CO_2-eq/ha (14.4 g CO_2-eq/MJ ME fed). Total costs were 251 €/t DM (23.8 €/GJ ME fed) for pit and 252 €/t DM (23.7 €/GJ ME fed) for baled silage. Three-cut RC produced 1.69 t CO_2-eq/ha for pit and 1.74 t CO_2-eq/ha for baled silage, at a total cost of 267 €/t DM and 258 €/t DM, corresponding to 24.5 and 25.4 €/GJ ME fed, respectively. For the conditions considered, the most favourable combined economic and environmental performance was observed for RC two-cut pit silage WS. The least favourable economic performance was associated with PRG two-cut baled silage WO, which showed the highest costs per hectare and per unit of energy fed. The highest GHGE per MJ ME fed was observed for PRG two-cut pit silage WO. Compared with PRG, RC reduced GWP by over 50%, while maintaining competitive or lower costs per unit of energy. Overall, systems with higher harvest frequency and greater input use increased GHGE and costs per hectare, while differences per ME fed remained limited. Integrating economic and GHGE metrics highlights how functional unit selection shapes mitigation assessment in grassland systems.

Constraints on greenhouse gas emission mitigation options of dairy cow farms in Switzerland through production systems and agro-climatic zones

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Reducing greenhouse gas (GHG) emissions of ruminant production systems is relevant for agricultural research and policy in Europe. However, most farm level mitigation measures do not exhibit strong effects and not all measures are applicable on all farms. Within the framework of the KlimaStaR Milch project, dairy farms in Switzerland are being paid primes for reducing their GHG emission intensity (GEI; kg CO2e/kg FPCM). Here, we assess their achievements over four years and discuss possible constraints by production systems and agro-climatic zones. In total, 216 farms were classified into three groups according to production system and zone: conventional valley/hill (C-VH; 109 farms), conventional mountain (C-M; 78 farms) and organic-all zones (ORG; 30 farms). Differences in GEI between the baseline (aggregated values 2019-2021) and the year 2025 were calculated using the Swiss GHG-calculation model KLIR. Separate Spearman rank correlations between the change in input variables (covering production, herd management, feeding, farm manure storage and land use) and the change in GEI for each system-zone group was used to assess the most relevant input variables. After four years, C-VH farms reduced their GEI by -0.064±0.052 kg CO2e/kg FPCM as compared to the baseline. This decline was significantly greater than for C-M (-0.044±0.059; p=0.014) and O-AZ farms (- 0.037±0.049; p=0.015), respectively. No difference was observed in GEI between C-M and O-AZ (p=0.53) farms. C-VH farms featured ten variables significantly associated with the decline in GEI: allocation factor (AF) milk (ρ = -0.34), annual milk yield/cow (ρ = -0.54), first calving age (ρ = 0.24), total milk production/farm (ρ = -0.38), proportion of specialized dairy-breed (-0.32), feeding of 3-NOP (ρ = -0.40), MJ NEL/kg DM pasture feed (ρ = -0.23), g CP/kg DM of roughage other than grass (ρ = -0.21), proportion of covered slurry pits (ρ = -0.22), total hours on pasture per year (ρ = -0.26). C-M farms shared four of these: AF milk (ρ = -0.52), annual milk yield/ cow (ρ = -0.68), first calving age (ρ = 0.39) and total milk production/farm (ρ = -0.55). Four variables were associated with a reduction of GEI only for C-M farms: total farm size (ρ = -0.26), proportion of slurry in biogas digesters (ρ = -0.35), proportion of slurry in covered pits (ρ = 0.23) and the proportion of manure in biogas digesters (ρ = -0.27). Only three variables were associated with a reduction of GEI for O-AZ farms. The AF milk (ρ = -0.75) and annual milk yield/cow (ρ = -0.50) were significant for both other farm groups, too, whereas productive lifespan (ρ = -0.44) was only significant for O-AZ farms. As C-VH farms have the least constraints by regulations, climate and topography, their potential to intensify dairy production is the highest. Variables explicitly linked to dairy production intensity were hence strongly associated with GEI. Up to now, the feeding of 3-NOP is only promoted for farms feeding total mixed rations and prohibited in organic farming. Increasing the proportion of farm manure fermented in biogas digesters and covering slurry pits were relevant for all conventional farms. Surprisingly, these measures were not associated with a reduction of GEI on organic farms. As the potential for intensification is limited on organic farms, increasing the productive lifespan of dairy cows appears to be a more frequent strategy. There is not necessarily a causal relationship between the input variables and the reduction in GEI. Nevertheless, our results highlight a set of potential GHG mitigation measures on dairy farms. Some are more applicable to specific agro-climatic zones or production systems than others.

Exploring scenarios for integral sustainability of Dutch dairy farms towards 2030

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The Dutch dairy sector faces multiple sustainability targets, including targets for greenhouse gas emissions (GHG), ammonia emissions (NH3), water quality, and biodiversity. This study estimated effects of alternative development pathways and management interventions on technical, environmental and economic performance of Dutch dairy farms towards 2030. Six farm types representative of the Dutch dairy sector were modeled with DairyWise for a 2022 reference situation (R22). Three pathways for 2030 were explored: business as usual (B30); higher productivity with advanced techniques and land-sparing (Hi30); and lower productivity with a larger land base and land-sharing (Lo30). All pathways included a similar increase in herd size per farm, genetic progress, regulatory measures (e.g., ending derogation), increased drought and heat, and a national herd reduction. Environmental impacts were quantified using the Annual Nutrient Cycling Assessment (ANCA) model. In B30, N soil surplus, NH3 emissions, and GHG emissions (per kg FPCM) decreased by 16–46%, 17-37%, and 2-21%, mainly due to planned and implemented regulation and agreements towards 2030. Access to pasture, manure export, and energy use increased; permanent grassland area declined; and land use per kg milk remained stable. Relative to B30, NH3 and GHG emissions decreased further in Hi30, by an additional 4-27% and 2-29%, and in Lo30 by 5-25% and 2-29%. Both Hi30 and Lo30 reduced manure export relative to B30 and increased green-blue infrastructure and measures under the Dutch agri-environment scheme (ANLb). Lo30 provided additional benefits on most farm types for N soil surplus, energy use, home-grown protein, access to pasture, and permanent grassland; Hi30 showed predominantly unfavorable effects for these variables, except for a lower land use per kg milk. Relative to B30, labor income declined on 3 of 6 farm types in Hi30, and 4 of 6 in Lo30. At national scale, assuming equal national herd size, B30, Hi30 and Lo30 resulted in 32%, 48% and 37% lower NH3; 13%, 28% and 23% lower direct non-CO2 GHG; and 11%, 14% and -2% less domestic land use. Milk volume would change by -6%, 1% and -21%. A mixed implementation of pathways seems most promising for meeting national targets and improving the integral sustainability of the Dutch dairy sector towards 2030.

Relevance, Variability, Availability – Evaluation of German dairy farm data for GHG accounting

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On-farm greenhouse gas (GHG) accounting faces a variety of requirements. It should be complete within defined farm-level system boundaries and consistent with the national inventory. For reliability purposes, regarding e.g. legally compliant reporting along the value chain, input data should have sufficient data quality and must be verifiable. Yet, to reduce data collection effort, GHG accounting ideally makes best use of suitable, already existing farm-level data. Last but not least, the input data needs to be sufficiently specific to allow a mapping of possible mitigation measures. The majority of German dairy farms are already obliged to regularly record a substantial number of variables, e.g. animal numbers and movements, fertilisation and cultivation planning as well as farm accounting. More data might be available at farm level for operational management. The more relevant these data are to the GHG balance and its variability across farms, the more important it is to ensure good data quality for these variables at the farm level. Based on the national inventory methodology and common farm-level GHG accounting tools, we identify GHG accounting-relevant variables for dairy farms. We investigate their variability and assess its impact on the GHG emissions. Following this, we explore the availability of the identified variables across farms, considering regular mandatory data collections, e.g. for accounting, traceability and inventory data management as well as quality and performance analysis. The availability of further voluntary data across dairy farms is also assessed. We map our findings in order a) to outline potential farm-data integration in GHG accounting tools b) to inform about potential needs for robust secondary data, and c) to derive recommendations for on-farm data collection for reliable, feasible and verifiable carbon accounting at the farm-level.

Environmental assessment of french rainbow trout production : a regional analysis

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ENVIRONMENTAL ASSESSMENT OF FRENCH RAINBOW TROUT PRODUCTION: A REGIONAL ANALYSIS De Montmorillon Sophie*1, Aubin Joël2, Tocqueville Aurélien3, Van Cuyck Claire4, Bonneau Jeanne1, Dupont-Nivet Mathilde1, Pouil Simon1 1Université Paris-Saclay, INRAE, AgroParisTech, GABI, Jouy-en-Josas, France 2UMR SAS, INRAE, Institut Agro Rennes-Angers, 35042 Rennes, France 3ITAVI, F-75009 Paris, France 4CIPA, F-75010 Paris, France *Presenting author: sophie.de-montmorillon@inrae.fr Rainbow trout (Oncorhynchus mykiss) is the main farmed fish species in France (29,000 tons in 2023). Production is traditionally based on freshwater flow-through systems. Farms are widely distributed across the country, with major producing regions including Hauts-de-France, Nouvelle-Aquitaine, Bretagne and Normandie. Production objectives vary according to market outlets: restocking and recreational fishing (10% of volumes), export (17%), and domestic human consumption (73%). Although these orientations are primarily driven by economic factors, environmental performance has become an increasing societal concern. Life Cycle Assessment (LCA) is a holistic and standardized method for environmental assessment. It has been applied to trout production to estimate impacts under various zootechnical and geographical conditions (Aubin et al., 2009; Chen et al., 2015; Maiolo et al., 2021). A previous environmental assessment of French rainbow trout farming was conducted by Chen et al. (2015). The present study provides an updated assessment of the environmental performance of French rainbow trout production, with a focus on regional differences among the main producing areas. Twenty-six surveys were conducted between March and November 2025 on conventional freshwater flow-through trout farms. Representativeness was evaluated by comparing the geographical distribution of surveyed farms with national aquaculture inventories from 2020 and 2022 (Agreste). Data collection covered all inputs and outputs required over one reference production year. For each farm, an LCA was performed to quantify environmental impacts per ton of live-weight trout at the farm gate. Principal component analysis and inferential statistics were applied to LCA results to assess whether the production region influences farm-level environmental impacts of trout production. Feed production was identified as the dominant contributor across most impact categories. Significant regional differences were observed for Net Primary Production use and Water Consumption, reflecting distinct feed compositions and sourcing strategies among regions, together with regional river characteristics. Environmental impact values were significantly correlated with zootechnical performance, particularly feed conversion ratio. Overall, inter-farm variability in environmental impacts appears largely explained by zootechnical characteristics which appear to reflect region-specific commercial strategies and rearing practices. To investigate the influence of market orientation on environmental performance, future assessments should extend system boundaries beyond the farm gate to include post-farm operations. REFERENCES Agreste,Enquête aquaculture. Aubin et al., 2009. Journal of Cleaner Production 17, 354-361. Chen et al., 2015. Journal of Cleaner Production 87, 87–95. Maiolo et al.,2021. Journal of cleaner production 289.

Advancing Harmonised Data Collection and Product-Based Greenhouse Gas Accounting in Dairy Production

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Farm-level greenhouse gas (GHG) accounting is becoming increasingly important in dairy production due to policy requirements, climate targets and market-driven sustainability standards. Within the RindforNet-SH network, we evaluated potential for standardized and digital data collection on German dairy farms and calculated product carbon footprints (PCF) for four German Holstein dairy research farms (conventional and organic; differing in housing, feeding intensity and herd structure) over the years 2022–2024. Emissions were calculated using the calculation standard for individual farm-level GHG accounting (BEK) from 2021 (KTBL 2021, 2nd ed. [1]). The calculation sheets and procedures are completely transparent and thus allow traceable calculations and adaptations. Digital interfaces (e.g., HIT (Animal traceability and information system), LKV (national milk inspection data) and on-farm weighing systems) were combined with equal pre-calculations to generate comparable datasets across farms. Farm-specific PCFs were calculated where possible, e.g. on farm forage production, while for other parameters standardized KTBL default values were used, e.g., imported concentrate feed. Average carbon footprints for milk ranged from 0.6 to 1.0 kg CO_2eq per kg ECM between farms. Year-to-year variation within farms was considerable and mainly driven by fluctuations in milk yields and forage yields. Higher milk yield per cow was associated with lower emissions per kg ECM, reflecting dilution of maintenance emissions. However, differences in forage quality and composition, replacement rate and manure management influenced total and product-based emissions. The BEK substitution approach for allocation of GHG emissions to milk and meat generated notable emission credits for culled cows and calves, resulting in considerably lower PCFs compared to using a biophysical allocation method. While the use of standard emission factors improves practicability and comparability, it reduces sensitivity to farm-specific mitigation strategies, particularly in forage production and youngstock rearing. Data quality proved to be a central determinant of robustness. Relevant uncertainties arose from discrepancies between produced and marketed milk, estimation of milk fed to calves (especially in cow–calf systems), and the reliance on default emission factors. Particularly forage production (yields and quality) emerged as key leverage point for mitigation and for farm specific improvements on data quality. This was especially important when grazing was involved due to large uncertainties in assessing the amount of feed intake during grazing in mixed systems including indoor feeding. This underlines the need for precise, farm-individual data and the need for further research into practical and precise assessment of forage yields and feed intake during grazing on farms. Digital data interfaces substantially reduced workload in data generation but revealed structural gaps, particularly regarding feed intake documentation, internal material flows and consistent recording across software systems. In conclusion, standardized yet flexible data frameworks combined with digital interfaces enable comparable and robust PCF calculation for dairy farms. However, methodological assumptions and data quality strongly influence results and mitigation assessment. Farm-individualized forage and herd data are essential to unlock reliable reduction potentials while maintaining practical feasibility for widespread application. [1] Arbeitsgruppe-BEK, Berechnungsstandard für einzelbetriebliche Klimabilanzen (BEK) in der Landwirtschaft. Handbuch, Darmstadt, Kuratorium für Technik und Bauwesen in der Landwirtschaft e. V. 2021.

The carbon footprint of Bonsmara cattle grazing Sourish mixed bushveld in South Africa

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The perception that beef production carries a larger carbon footprint compared with other foods, has a negative impact on the sector. It is therefore essential to obtain local data of the whole system to shed light on the soil-plant-animal interactions relating to the carbon balance of the system. The aim of the study was to establish a realistic baseline carbon footprint for extensive beef production under natural grazing conditions by taking into account enteric methane emissions as well as the grassland and soil carbon components. The study was conducted using 10 multiparous Bonsmara cows grazing approximately 50 ha of undisturbed rangeland in the Sourish mixed bushveld of South Africa. Stocking density was maintained at 0.2 LSU ha^{-1}. Methane emissions were estimated based on the enteric methane emission factor for commercial beef cattle in South Africa (78.9 kg CH_4 LSU^{-1} $year^{-1}$). Annual methane emissions per cow were converted to carbon dioxide equivalents (CO_2-eq) using the 100-year global warming potential (GWP^{100}). Gross seasonal carbon input from the rangeland was determined from above-ground dry matter production measured over the three growing seasons. Veld evaluation was done by both the step-point and quadrant veld evaluation methods. Above- and below-ground carbon stocks were derived using weighted root-to-shoot ratios for grass species, calculated according to their relative abundance in the veld. A carbon fraction of 0.45 was assumed for dry biomass. Dry-season contributions were considered negligible due to minimal plant growth. Net seasonal vegetation carbon was calculated as the difference between gross carbon input and estimated decomposition losses. Soil organic carbon (SOC) stocks were determined to a depth of 60 cm from measured SOC concentration and bulk density. Soil respiration was measured during the growing season to quantify CO_2 release from soil. The seasonal carbon emission fraction (f-emission) was calculated as the ratio of measured soil respiration C-flux to total estimated decomposed carbon, estimating the proportion of decomposed biomass carbon released to the atmosphere. Net seasonal carbon balance (soil + vegetation) was converted to CO_2-equivalents using the IPCC (2006) molecular mass ratio (44/12). Total system CO_2-eq per hectare, including cattle methane emissions combined with soil and vegetation carbon fluxes over a 12-month period, was then calculated. All procedures involving animals were approved by the ARC-API Ethics Committee (APIEC21/12) and conducted under Section 20 authorisation of the Animals Protection Act No. 71 of 1962 (Ref nr 12/11/1/1/15 (2167JD)). The total carbon footprint of the system, including enteric methane emissions from the animals and measured soil and vegetation carbon losses, was approximately 20.5 kg CO_2eq kg^{-1} live weight per year. The total system CO_2eq per ha over a 12-month period is calculated at 244kg CO2eq ha^{-1}. The carbon footprint measured in this study is therefore in line with published values for extensive, low-input rangeland systems under semi-arid conditions. The results show that under semi-arid South African conditions, an extensive beef production system can maintain near carbon-neutral dynamics within the rangeland.

Pre- and post-weaning management and nutritional strategies to increase piglet growth and health

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At weaning piglets are confronted with abrupt changes to their physical and social environment. This is accompanied by a growth check and frequently by post-weaning diarrhoea in piglets. Increasing litter size has increased within-litter piglet weight disparity and reduced mean piglet weaning weight, thereby exacerbating the challenge. A number of management and nutritional strategies to increase pre- and post-weaning piglet growth and health and consequently improve post-weaning (PW) outcomes have been proposed. These are particularly important considering recent bans on the use of pharmacological levels of ZnO and in-feed antibiotics. Pre-weaning strategies such as improved pen hygiene, split-suckling, provision of pain relief to sows and creep feeding (in dry pellet and liquid form) can help to reduce mortality, increase weaning weight and improve PW outcomes. Immediately PW, strategies including improved pen hygiene, liquid feeding, increased floor and feeder space allowance and feeding diets with reduced acid binding capacity (ABC) can help to increase PW growth, survival and lifetime growth. These pre- and post-weaning strategies have been evaluated by our group and are reported here. Implementation of an optimized farrowing house hygiene routine reduced the bacterial load in farrowing pens, which led to reduced diarrhoea prevalence and clinical cases of disease, as well as antibiotic and anti-inflammatory usage; all culminating in increased piglet weaning weight. However, intensive split-suckling reduced pre- and post-weaning average daily gain (ADG) and did not impact medication usage. Postpartum administration of the non-steroidal anti-inflammatory drug, meloxicam, to sows is an inexpensive and easily implemented strategy. It reduced clinical cases of disease and medication usage and increased ADG during the first two weeks of life and early PW, culminating in increased final carcass weight. Pre-weaning, liquid feeding increased weaning weight and reduced pre-weaning mortality but had no residual effect on PW growth. Provision of supplemental milk replacer for 10 days PW dramatically increased average daily feed intake and ADG and final carcass weight. However, milk replacer is expensive and its use must be economically justified. Good pen hygiene is considered essential during the PW period; however, optimising PW pen hygiene over that achieved with a basic hygiene protocol conferred no additional growth or health benefits. Doubling the legal floor space allowance during the PW period increased piglet growth by 7.3% to 45 days PW and at this floor space allowance increasing feeder space allowance improved feed conversion efficiency (FCE) by 6.3% between day 27 and 45 PW. Feeding diets without limestone flour for 14 days PW so that their ABC-4 was reduced by 130 meq/kg to ~300 meq/kg increased ADG and improved FCE. Reducing dietary ABC-4 by 230meq/kg to ~ 200meq/kg via dietary inclusion of fumaric acid, during the same period, also improved FCE. In summary, a number of successful management and nutritional strategies exist to improve pre- and post-weaning piglet growth and health. However, strategies should be selected to suit individual production systems and must always be cost-beneficial.

Delayed weaning impacts on piglet gut physiology and metabolism

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Weaning is defined as the transition from milk to solid feed intake. Under free-range conditions, this transition is gradual from the initiation of solid feed intake between 2 and 3 weeks of age until milk intake ceases completely between 10 and 17 weeks of age. This contrasts with usual practices in conventional pig farming, where weaning is carried out much earlier, between 3 and 4 weeks of age. At this stage, voluntary solid feed intake is very low and the digestive system still immature, making this feed transition to exclusive solid feed very abrupt. This period is often associated with digestive disorders that adversely affect piglet health, leading to increased medication use and a decline in technical and economic performance. The impact of weaning age has been extensively studied in the literature up until 35 days of age, but the effects of delayed weaning in pig farming (>40 days of age), closer to the conditions observed in free-range situations, have been poorly documented, although this practice is becoming more widespread, mainly in organic farming, where specifications require weaning at over 40/42 days of age in the EU. Thus, our study aimed to assess impacts of delayed weaning (49 vs 21 days of age) on the gut physiological and metabolic adaptations of piglets. (Large White x Landrace) x (Pietrain x Duroc) piglets from 10 litters were studied. Within each litter, at 21 days of age, piglets were separated into two groups: conventional weaning at 21 days of age (n=3 per litter, W21, fed exclusively with solid feed) vs delayed weaning at 49 days of age (W49, remaining litter with access to maternal milk until 49 days of age and solid feed). In order to evaluate early and delayed impact of weaning age, 9 piglets per group (sex ratio 50:50, paired within litter with equivalent weight at 21 days of age) were euthanized at 28 and 56 days of age and colon tissue and content were sampled. Microbial response was evaluated through metabolomic analyses by NMR and metagenomic analyses by 16S rDNA sequencing. Host response was evaluated through IgA level measurements and transcriptional analyses by RNA sequencing (RNA-seq). Suckling piglets (W49) were heavier with a shorter colon than weaned piglets (W21) at 28 days of age, while no differences persisted at 56 days of age once all piglets were weaned. The composition and activity of the colon microbiota were altered by weaning age. Colon pH was higher in suckling piglets (W49) associated with a higher level of amino acids and branched chained fatty acids, while short chain fatty acid levels were unaffected by weaning age at 28 days of age. At 56 days of age, no impact of weaning age on pH was observed and modulations of microbial metabolic activity were weak. When looking into the host response to weaning, no impact was observed on IgA levels regardless of age. RNA-seq data evidenced 243 genes that were modulated by weaning age at 28 days of age while solely one was modulated at 56 days of age. Genes involved in mitochondria metabolism, BCAA degradation and absorptive cell differentiation were differentially expressed between suckling and weaned piglets at day 28. Our study showed that weaning age had strong early impacts on gut physiology and metabolism but only weak impacts persisted in later stages of life. These results will be integrated with behavioral evaluations of the piglets and the sows in order to have a global view of the impact of delayed weaning in pig farming.

A fresh start: The effect of hygiene routine in the farrowing room on piglet faecal consistency, performance, and tail damage prevalence

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Poor hygiene practices in pig production can lead to reduced growth and an increased risk for disease and damaging behaviour. On the other hand, early-life exposure to sow faeces may support piglet gut microbiome maturation and growth. The objective of the current trial was to evaluate the effects of three hygiene routines in the farrowing room: 1) An incomplete hygiene protocol (water wash) + removal of sow faeces (W); 2) A complete hygiene protocol including washing and disinfection + removal of sow faeces (D); 3) Complete hygiene protocol + no removal of sow faeces (F). Sow faeces was removed twice daily (except for treatment F) for the first 2 weeks post-farrowing. Floor swabs were collected from each farrowing pen before the sows entered the farrowing room to verify differences in hygiene status between hygiene protocols. Each treatment consisted of 16 litters with intact tails (15 piglets/litter), divided over two batches. Piglets were weaned at 29 ± 0.1 days of age, weighing on average 8.4 ± 0.11 kg. All weaner pens were cleaned according to the complete hygiene protocol. After weaning, piglets were mixed within pre-weaning treatment and all pigs were housed and managed similarly, with 18 pens (6 piglets/pen) per treatment group. Pig performance and faecal consistency were analysed using a mixed model with pen as experimental unit. Diarrhoea occurrence and tail damage were scored on individual level and analysed according to a GLM using a multinomial distribution and logit transformed data. Floor swabs showed higher adenosine triphosphate (ATP) levels (W: 1.9 vs. D: 1.1 log10 reflective light units/cm_2; P<0.001), a higher aerobic total bacterial count (W: 931 vs. D: 42; P<0.001) and higher enterococci levels (W: 40.3 vs. D: 7.6; P = 0.01) for the farrowing pens cleaned according to the incomplete versus complete hygiene protocol. These results indicate reduced cleaning efficacy as a result of the incomplete protocol, reflected by increased organic residue and higher bacterial and faecal contamination. No differences were found between the three treatment groups in the farrowing room in terms of faecal consistency, diarrhoea prevalence, litter size at weaning, growth or weaning weight. W-piglets had a higher pre-weaning intake of solid feed than F-piglets (17 vs. 11 g/piglet/d; P=0.04) and a higher prevalence of tail damage at 2 weeks of age (2.6 vs. 0.4%; P = 0.02). Post-weaning morbidity (6 piglets) and mortality (1 piglet) was low. After weaning, W-piglets ate and grew faster than D-piglets in weaner phase I and II, as well as the total period (ADFI: 563 vs. 523, P<0.01; ADG: 445 vs. 404; P<0.001). F-piglets performed similarly as D-piglets in phase I, grew similarly as W-piglets in phase II, and had intermediate growth in the total period. At D28 post-weaning, W- (+1.1 kg) and F-piglets (+0.7 kg) weighed more than D-piglets (P<0.01). No significant effects on feed efficiency or faecal score were observed after weaning (P>0.05). At 2 weeks post-weaning, W-piglets had the highest prevalence of tail damage (W: 7%, D: 4%, F: 0%; P<0.001). Results of the current study suggest that early microbial exposure may influence both behavioural development and post-weaning performance. Analysis of the piglets' microbiome from rectal swabs remains to be conducted, and may help to explain the observed differences among the experimental groups. This research is part of PPP-project Gut2Go, which receives 50% of its funding from public sources through the TKI Agri & Food programme (Top Consortium for Knowledge and Innovation in Agri & Food).

Pre-Weaning Supplementary Milk Enhances Post-Weaning Feeding Behaviour and Growth Performance in Piglets

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Early feed intake after weaning is critical for piglet health and performance, yet heavier piglets often show delayed adaptation to solid feed. This study evaluated the effects of pre-weaning supplementation with a complementary milk on post-weaning feeding behaviour and growth. Twenty-six sows were assigned to either a control group or a group whose litters received complementary milk replacer (Danmilk Supreme® via the Pump'n'Grow cup system, AB Neo) from day 7 to 18 of lactation. All litters received a prestarter diet from d14 of lactation to weaning. From each litter, the two smallest (S; excluding stunted/IUGR pigs) and two heaviest (H) piglets were selected and eart-agged the day before weaning, resulting in a 2 × 2 design: small vs. heavy piglets and complementary milk (Milk) vs. control (Con). At weaning (day 24), piglets were transported to an experimental facility equipped with an electronic feeding system that continuously recorded individual feed intake, water intake, and body weight. Each group was assigned to a single pen and all pigs had ad libitum access to the same prestarter diet offered in the farrowing house. However, unequal group sizes and body weights occurred because some ear-tagged piglets could not be located within the full batch after transport to the nursery site, particularly in the small-weight category (S+Milk: 13 pigs, 6.1 ± 0.81 kg of body weight (BW); S+Con: 21 pigs, 4.9 ± 1.04 kg BW; H+Milk: 25 pigs, 8.25 ± 0.83 kg BW; H+Con: 22 pigs 8.8 ± 1.21 kg BW). During days 0–14 post-weaning, milk-supplemented pigs displayed numerically higher average daily gain (ADG) than their respective controls, with S+Milk outperforming both S+Con and H+Con groups (S+Milk: 184 g/d^{a}; S+Con: 98 g/d^{b}; H+Milk: 151 g/d^{ab}; H+Con: 108 g/d^{b}; $p = 0.002$). During this period, average daily feed intake (ADFI) was also higher in Milk-supplemented pigs than in controls (S+Milk: 222 g/d^{a}; S+Con: 157 g/d^{b}; H+Milk: 219 g/d^{a}; H+Con: 152 g/d^{b}; $p = 0.047$). Across the 42-day post-weaning period, S+Milk pigs showed greater ADFI ($p = 0.017$) than S+Con pigs (603g/d vs 461g/d) and both Milk-supplemented groups showed a tendency for improved growth compared with their respective control groups (S+Milk: 407 g/d^{x}; S+Con: 334 g/d^{y}; H+Milk: 398 g/d^{x}; H+Con: 352 g/d^{y}; $p = 0.066$). Marked differences in early feeding behaviour were observed. Small pigs receiving DanMilk exhibited the shortest latency to first feed (mean= 7.7 h), compared with S+Con (12.3 h), H+Milk (15.9 h), and H+Con (27.6 h). The time-to-event analysis confirmed that H+Con pigs required more time to initiate feeding than both small pig groups ($p < 0.05$). During the 24-hour period following each pig's first feeding event, S+Milk pigs consumed more feed (S+Milk 213g^{a}, S+Con 153g^{ab}, H+Milk 127g ^{ab}, H+Con 78g ^{b}; $p = 0.003$) and performed more feeder visits involving feed consumption (S+Milk: 10.8^{a}, S+Con 6.5^{ab}, H+Milk 6.6^{ab}, H+Con 4.4^{b}; $p = 0.006$) than H+Con pigs. Overall, pre-weaning milk supplementation (Danmilk Supreme®, AB Neo) improved early post-weaning feeding behaviour and supported enhanced growth in both small and heavy pigs. Small pigs consistently demonstrated faster adaptation to solid feed than heavy pigs, with the greatest benefits observed in Milk-supplemented small pigs. Interpretation should, however, consider confounding effects from unequal starting body weights and group sizes, particularly within the small – milk supplemented group.

Dietary supplementation with sodium butyrate and plant extracts to optimize the weaning process and productive outcome in piglets

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The post-weaning period in piglets is characterized by gastrointestinal immaturity, microbial instability and inflammatory responses, which may impair nutrient utilization and intestinal homeostasis. Following the ban of antibiotic growth promoters and zinc oxide, alternative nutritional strategies are needed. Several nutritional approaches, such as butyrate or plant extracts, have shown promising results when used individually, however their potential combined effects remain largely unexplored. The objective of this study was to investigate the effects of dietary supplementation with sodium butyrate (BU), a plant extract blend (PE) containing turmeric, green tea, Scutellaria baicalensis and sodium gluconate, and their combination as strategies to optimize the post-weaning in piglets. A total of 120 male piglets (22–25 d of age; 6.8±1.2 kg) were randomly allocated to four dietary treatments in a 2×2 factorial design: control (BU−PE−), sodium butyrate (BU+PE−), plant extract (BU−PE+), and their combination (BU+PE+), with five pens per treatment with homogeneous animal size. Additives were included at 3 g/kg in the pre-starter diet (day 1-14 post-weaning) and 2 g/kg in the starter diet (d 15–44). Growth performance, diarrhoea incidence and morbidity were recorded weekly. Feed digestibility, blood biochemical and cytokine profiles, gastrointestinal fermentation parameters, and ileal histomorphology were assessed at the end of each phase. Results showed that during the pre-starter phase, PE supplementation increased nutrient digestibility (+1.2% for DM, +1.7% for CP, + 3.0% for EE and +12% for NDF, $P < 0.05$). Supplementation with BU reduced blood concentration of pro-inflammatory cytokines such as IL-8, GM-CSF and IFNγ. The combination of both additives tended to decrease the concentration of IL-6, suggesting a superior intestinal health. Regarding gut fermentation, BU supplementation increased total VFA concentration in the stomach, particularly butyrate. Supplementation with either additive alone increased ileal ammonia-N concentration ($P = 0.025$), whereas PE supplementation increased total VFA concentration in the ileum ($P = 0.024$) and decreased the proportion of branched-chain fatty acids in the cecum (-42%). Histologically, PE supplementation increased the villus height and papilla surface area in the ileum. During the starter period, most of the previously observed effects tended to disappear. Piglets supplemented with BU alone had higher nutrient digestibility (+3.0% for DM, +3.6% for CP, +14.7% for EE a +10.2% for NDF, $P < 0.05$) compared with their un-supplemented counterparts. Moreover, BU supplementation increased blood glucose concentration (+11%), whereas PE supplementation increased total proteins and albumin concentrations, indicating improved energy and protein uptakes, respectively. Blood inflammatory markers, ileal histology and gut fermentation parameters remained largely unaffected during the starter period. Piglets supplemented with BU tended to have a higher VFA concentration in the cecum (+19.8%, $P = 0.080$), which may explain the improved digestibility. In conclusion, this study demonstrated that post-weaning adaptation can be optimized through nutritional interventions. Supplementation with BU reduced inflammatory markers, whereas PE enhanced intestinal epithelium morphology and feed digestibility during the first two weeks after weaning. The combination of both additives did not result in additional improvements, contrary to the initial hypothesis. Further studies are warranted to explore the productive implications under more challenging conditions.

Propionic acid–preserved grain enhances growth performance more effectively than combined butyric acid and resistant starch supplementation in weaned pigs

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Weaning represents a critical transition in commercial pig production and is commonly associated with reduced feed intake, immature digestive capacity and instability of the intestinal microbiota, predisposing piglets to post-weaning diarrhoea and impaired growth performance. Nutritional interventions capable of supporting gut function and maintaining performance during this period are therefore of considerable interest. Preservation of grain using organic acids such as propionic acid (PA) may improve feed hygiene, nutrient utilisation and intestinal function. Alternatively, dietary supplementation strategies targeting butyrate supply may enhance epithelial health, either directly through butyric acid (BA) inclusion or indirectly through microbial fermentation of resistant potato starch (PS). The present study evaluated the independent and combined effects of PA-preserved grain and BA + PS supplementation on growth performance, intestinal microbiota composition and hindgut fermentation characteristics in weaned pigs. A total of 144 piglets (28 days of age) were allocated to one of four dietary treatments in a 2 × 2 factorial arrangement consisting of dried grain or PA-preserved grain (4 g PA/kg grain) with or without supplementation of BA (1.5%) and PS (1%). Growth performance was monitored throughout the post-weaning period through measurements of average daily feed intake (ADFI), average daily gain (ADG) and gain-to-feed ratio (G:F). Faecal consistency was assessed twice daily using a 1–5 scoring system to evaluate diarrhoea incidence. On day 8 post-weaning, intestinal tissue and digesta samples (n = 12) were collected for assessment of intestinal morphology, microbial composition and volatile fatty acid (VFA) concentrations. Feeding PA-preserved grain increased ADFI, ADG and final body weight compared with dried grain diets ($P < 0.05$), despite higher dietary deoxynivalenol concentrations. PA-preserved grain also altered hindgut microbial composition, increasing the relative abundance of several fibre-fermenting and butyrate-associated bacterial genera, alongside increases in proteolytic bacterial taxa and colonic branched-chain fatty acid concentrations ($P < 0.05$). These changes were accompanied by a higher incidence of diarrhoea compared with pigs offered dried grain diets ($P < 0.05$). Across grain types, BA + PS supplementation increased the abundance of butyrate-producing bacteria, including Roseburia and Faecalibacterium, and increased total VFA concentrations in caecal digesta ($P < 0.05$). However, BA + PS supplementation did not influence growth performance or faecal scores ($P > 0.05$). A significant grain × supplementation interaction was observed for G:F ($P < 0.05$), whereby BA + PS improved feed efficiency in pigs fed dried grain but not in those receiving PA-preserved grain. In conclusion, PA-preserved grain exerted the strongest overall effects on post-weaning growth performance and hindgut fermentation, indicating its potential as an effective nutritional strategy to support piglet performance after weaning. Supplementation with BA + PS may provide complementary benefits in conventional dried grain diets through enhancement of butyrate-producing microbial populations and improved feed efficiency, although additive effects were not observed when combined with PA-preserved grain.

Berry Good for the Gut: The effect of Aronia melanocarpa and Ribes nigrum Pomace Mix on Oxidative Stress and Gut Health in Weaned Pigs Challenged with Enterotoxigenic Escherichia coli F18

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During the weaning transition, pigs face multiple stressors, such as abrupt dietary changes, housing transitions, and infections with enteric pathogens such as enterotoxigenic Escherichia coli (ETEC), which collectively cause oxidative stress and increase the risk of developing postweaning diarrhea (PWD). To control PWD, pigs are often treated with antibiotics, however reducing antibiotic use is essential given the globally growing problem of antimicrobial resistance (AMR). Dietary antioxidant supplementation may mitigate oxidative damage and enhance endogenous antioxidant enzymes. Berry pomace, a by-product of juice production, is rich in bioactive compounds with antioxidative, anti-inflammatory, and antimicrobial properties, making it a promising nutritional strategy to support pig health and oxidative status during this critical transition. This study aimed to investigate the effect of supplementation with a mixed berry pomace derived from Ribes nigrum and Aronia melanocarpa on the oxidative status and gut health of pigs postweaning. 72 newly weaned 26-day-old pigs (body weight (BW) = 6.3 ± 1.48 kg) were randomly assigned to four dietary treatments: standard weaner diet (CON), CON + berry pomace (50 g/kg feed; MIX), CON + challenged with ETEC F18 (CON_ETEC), and MIX + challenged with ETEC F18 (MIX_ETEC). The experiment was conducted in two blocks, each lasting 17 days postweaning. Pigs were housed in mixed-litter, mixed-sex pens of three. On days 4 and 5 postweaning, piglets in MIX_ETEC and CON_ETEC received an ETEC inoculum orally (5 ml of 10^9 CFU/ml; strain O138 F18-ETEC 9910297-2STM), while CON and MIX received phosphate-buffered saline. Feed intake was recorded daily, BW weekly to calculate average daily feed intake (ADFI), average daily gain (ADG), and gain to feed ratio (G:F). Fecal samples were collected every second day to assess diarrhea occurrence. On day 10 postweaning, one pig from each pen was euthanized, and tissue samples from the small intestine (SI; at 50% length) were collected for analysis of oxidative stress biomarkers (malondialdehyde (MDA) and superoxide dismutase (SOD)). The remaining two pigs in each pen were euthanized on day 17 postweaning, and SI samples were collected for MDA and SOD. Pigs from groups supplemented with berry pomace (MIX and MIX_ETEC) had a higher BW on day 17 ($p < 0.05$) compared to CON and CON_ETEC. MIX tended to have a higher ADFI from day 11 to 17 postweaning ($p = 0.09$) compared to CON. MIX tended to have a lower G:F from day 11 to 17 postweaning ($p = 0.06$) compared to CON, but had no statistical difference in ADG. No statistical differences in ADFI, ADG, or G:F were observed between CON_ETEC and MIX_ETEC. MIX_ETEC tended to have a higher fecal DM from days 10 to 14 postweaning ($p = 0.097$) compared to CON_ETEC. MDA concentrations in SI tissue did not differ between MIX and CON on day 10 or 17 postweaning. MIX_ETEC had a lower MDA concentration in SI tissue on day 10 ($p = 0.01$) compared to CON_ETEC; however, no difference was observed on day 17 postweaning. Regardless of the ETEC challenge, no difference in SOD activity was observed in SI tissue on either day 10 or day 17 postweaning. In conclusion, diets supplemented with berry pomace mitigate oxidative stress biomarkers, shown by reduced SI tissue MDA concentration in challenged pigs compared to control pigs, and indicate faster post-ETEC recovery in pigs receiving berry pomace compared with controls. This project is part of the PIG-PARADIGM project, funded by the Novo Nordisk Foundation under grant number NNFSA210073688.

Impacts of encapsulated amino acids on growth performance, gut microbiota, and the caecal metabolome in weaned pigs

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Piglets encounter post-weaning challenges that significantly affect growth performance and gut health. Amino acids (AAs) can be considered potential prebiotic able to modulate gut microbiota and serve as precursors for functional molecules potentially enhancing animals'robustness. Their encapsulation could increase their availability in the hindgut where microbes are abudant. This study evaluated the effects of encapsulated AAs on growth performance, cecal metabolome and metagenome of weaned pigs. A total of 168 weaned pigs (28 days old), housed in 56 pens, were assigned to seven dietary treatments: 1) a Control group (standard weaning diet) and six groups receiving encapsulated AAs: 2) lysine (Lys, 2 kg/t), 3) tryptophan (Trp, 2 kg/t), 4) branched-chain AAs (BCAA, 2 kg/t), 5) combination of Lys (1 kg/t) and Trp (1 kg/t) (Lys + Trp), 6) combination of Lys (1 kg/t) and BCAA (1 kg/t) (Lys + BCAA), 7) combination of Trp (1 kg/t) and BCAA (1 kg/t) (Trp + BCAA) on top to the basal diet. Body weight (BW), average daily gain (ADG), feed intake (FI), and feed-to-gain ratio (F:G) were recorded weekly. At d14 and d28, one piglet per pen was euthanized for collecting gut content in ileum, caecum and colon. Free AAs were quantified in ileum content and faeces at day 28 by HPLC. Cecal microbiota was analyzed using shotgun metagenomics, and cecal metabolomics was performed by UPLC-MS/MS (Metabolon Global Discovery Panel) at day 28. Although BW and FI did not differ among treatments, ADG and F:G improved during days 21–28 in the BCAA and Trp + BCAA groups compared with the Control ($P < 0.05$). Free Trp concentrations were higher in the ileum of the Trp group compared with the Control ($P < 0.05$). However, Trp disappearance (ileal Trp – faecal Trp) did not differ among groups, suggesting targeted release and utilization. Global microbial diversity indicators did not differ across treatments, however metagenomic analysis revealed an increased relative abundance of Kahnovirus in the Trp group, Flintibacter sp. KGMB00164 in the BCAA group, and Roseburia hominis in the Trp + BCAA group (LDA > 3, $P < 0.05$). For metabolomics, the two-component sparse partial least squares discriminant analysis (sPLS-DA) model (Balanced error rate = 0.78) showed clear separation of BCAA and Trp from the Control. Component 1 was mainly driven by lipid metabolites, particularly glycerophospholipids, discriminating the BCAA group. Pathway analysis confirmed significant enrichment of lysophospholipid and long-chain polyunsaturated fatty acid metabolism ($P < 0.001$). In contrast, Lys was associated with metabolites linked to glutathione metabolism ($P < 0.01$), indicating modulation of redox pathways. Component 2 primarily differentiated the Trp group through nitrogen-related metabolites, with enrichment of sphingomyelin and dinucleotide metabolism ($P < 0.05$). The Trp+BCAA group also showed shifts in lysophospholipid ($P < 0.01$) and pyrimidine metabolism ($P < 0.05$). Encapsulated AAs, particularly BCAA and Trp + BCAA, improved ADG and F:G during the last experimental week (d21–28). Beyond this period-specific effect, they modulated the gut microbial ecosystem and cecal metabolic environment, especially lipid, nitrogen, and redox pathways. These results suggest that encapsulated AA can influence microbial and metabolic functions in weaned pigs, potentially supporting post-weaning robustness.

Effect of increasing dietary lysine level on transcriptomic profile in pig longissimus thoracis muscle following a period with dietary protein restriction after weaning

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Lysine (Lys) is a building block for muscle protein synthesis and the most limiting amino acid (AA) in weaning diets. It is unclear how increasing dietary Lys levels affects molecular mechanisms in muscle, after dietary crude protein (CP) restriction during the early weaning period. Low protein (LP) pigs were fed a CP/essential AA (EAA)-restricted diet (−20% CP vs. standard (STD) CP level) during weeks 1–4 after weaning (W1–4). During weeks 5–8 after weaning (W5–8), pigs received diets with dietary Lys and other EAA increased by 10% (LP-STD10+, N = 12) or 20% (LP-STD20+, N = 12) above the standard level (LP-STD, N = 12) to evaluate the effect of increasing AA levels. A control group (STD-STD, N = 12) was not restricted in dietary CP during W1–4 and received a standard CP diet during W5–8. At the end of W8 all pigs were euthanized and tissue samples from the longissimus thoracis muscle were collected for transcriptomic profiling by RNA-seq. Differential gene expression was analysed using DESeq2 (R Project), and pathway enrichment evaluated using gene set enrichment analysis (GSEA; Broad Institute). Results were considered significant at adjusted $p < 0.05$ (DESeq2) and $q < 0.05$ (GSEA). In LP-STD vs. STD-STD pigs, the metabolic adaptation genes FGF21 and PDK4, alongside HABP2, were up-regulated ($P < 0.05$), and the metabolic, immune and nutrient regulating genes SSH3, ARG1 and STRA6, were down regulated ($P < 0.05$). A significant suppression ($P < 0.05$) of pathways related to general metabolism, steroid hormone biosynthesis, and AA metabolism was observed in LP-STD vs. STD-STD pigs. On the contrary, the oxoacid and organic acid metabolic process and apical plasma membrane pathways were relatively enriched ($P < 0.05$) in LP-STD vs. STD-STD pigs. In LP-STD10+ and LP-STD20+ vs. LP-STD pigs, the immune regulating genes TREM2, BTK and DOCK2, and CD14, CCR1 and NCF1 were up regulated ($P < 0.05$), and the AA metabolism genes ASNS, SLC7A5, and MIOX, and FGF6, ARG2 and PSAT1 were down regulated ($P < 0.05$). In LP-STD10+ and LP-STD20+ vs. LP-STD pigs, the cytokine-cytokine receptor interaction and chemokine signalling, and B cell receptor signalling pathways were activated ($P < 0.05$), while the regulation of immune system process pathway was relatively enriched ($P < 0.05$). On the contrary, biosynthesis of AA was suppressed ($P < 0.05$) in both LP-STD10+ and LP-STD20+ vs. LP-STD pigs. In LP-STD10+ and LP-STD20+ vs. STD-STD pigs, genes related to metabolic regulation and stress signaling (TXNIP, SOG3, PDK4) and to apoptotic/tissue-response pathways (ANXA8, DEDD2, DMRTA1) were upregulated ($P < 0.05$), while metabolic genes PSAT1, QPRT, PLPP4, and PAPPA, FGF6 and TOPAZ1 were down regulated ($P < 0.05$). In LP-STD10+ and LP-STD20+ vs. STD-STD pigs, GSEA analysis showed the inflammatory mediator regulation of transient receptor potential channels pathway, and chemokine signalling pathway was activated, while pathways involved in oxoacid and organic acid metabolic process, and icosanoid transmembrane transporter activity and xenobiotic transmembrane transporter activity were relatively enriched ($P < 0.05$). The results suggest that increasing the dietary Lys and other EAA levels after a dietary CP restriction modulates muscle metabolism, with potential implications for health and performance.

Supplementation of His and Arg during the nursery phase improves gut health in weaning piglets fed low crude protein diets.

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Post-weaning diarrhea (PWD) is still a major challenge in the swine industry, causing mortality and growth retardation in piglets. In front of the recent ban of ZnO, reducing dietary crude protein (CP) level can be a useful strategy to control PWD. However, growth performance may be impaired despite balancing essential amino acids (AA). Histidine (His), an essential AA, and arginine (Arg), a conditionally essential AA, are involved in important metabolic pathways associated with immune response and intestinal health and may help to enhance gut function under low CP feeding conditions. Fecal calprotectin has been shown to be a non-invasive biomarker of intestinal inflammation in piglets. Therefore, it was hypothesized that providing His and Arg above nutrient requirements would benefit weaned piglets fed low CP diets. This study evaluated the effects of 38% SID His:Lys and 95% SID Arg:Lys ratios on growth performance and gut health parameters. Distributed in two batches, 552 weaning piglets (DanBred × Pietrain; initial BW 6.2 ± 0.67 kg) were randomly allocated into 23 mixed-sex pens (12 pigs/pen). Four diets were offered from 0 to 15 days (phase 1) and two from 16 to 42 days (phase 2). Dietary treatments of phase 1 were arranged in a 2×2 factorial design with CP level (14% vs 16%) and the addition or not of His and Arg (no vs FAA) as main factors. All diets were balanced with all essential crystalline AA according to the FEDNA (2013) guidelines. His and Arg were supplemented to achieve 38% SID His:Lysine (Lys) and 95% SID Arg:Lys ratios in FAA treatments. During phase 2, two diets of 18%CP with or without FAA were offered according to the previous treatments. BW and FI were assessed on days 0, 15, 28 and 42 of the trial. ADG, ADFI ang G:F ratios were calculated for each period. On day 15 blood and fecal samples were collected for analysis of serum urea nitrogen (SUN) and calprotectin. Fecal score was registered every 2 days according to Hu et al (2012). Data were analyzed using the lmer package of the R software considering CP level, FAA and their interaction as fixed effects and batch included as a random effect. No CP*FAA interaction was detected ($P>0.05$). When significant effects were observed, post hoc comparisons were performed using Tukey's test with R software. Increasing CP level positively affected growth performance parameters during phase 1, with higher BW ($P<0.05$), ADG ($P<0.01$) and G:F ratio ($P<0.01$). SUN concentrations tended to decrease with CP reduction ($P=0.080$), suggesting a limitation in nitrogen availability in 14%CP diets despite adequate AA balance. In contrast, the increased ratios of His and Arg did not markedly affect growth but tended to increase ADFI ($P=0.089$) and reduce diarrhea incidence during phase 1 ($P=0.073$) and the overall experimental period ($P=0.061$) which was supported by the lower concentrations of fecal calprotectin ($P<0.05$). In conclusion, SID 38% His:Lys and SID 95% Arg:Lys ratios showed beneficial effects on gut health parameters while maintaining growth performance in piglets fed low CP diets. These results highlight the functional roles of these amino acids in mitigating PWD under protein-restricted conditions.

Impact of a bioactive additive as an alternative to antibiotic growth promoters on growth performance, gut health, and inflammatory response in weaning piglets

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After weaning, piglets face physiological, nutritional, and immunological challenges, which often lead to impaired gut health and weight loss. To mitigate these adverse effects, antibiotic growth promoters (AGPs) were introduced into animal production; however, their use has stimulated bacterial resistance. The search for alternatives is constant, with acidifiers and essential oils emerging as promising possibilities due to their antimicrobial and immunomodulatory effects. Thus, this study aimed to evaluate a microencapsulated bioactive additive composed of acidifiers (citric, fumaric, malic, and phosphoric acids) and essential oils (thymol and carvacrol) as an alternative to AGPs on the performance, gut health, and inflammatory response of weaned piglets. We hypothesized that, due to its positive properties, the bioactive additive would show equal or superior effects compared to the AGP. The 43-day experiment involved 120 piglets blocked by initial weight and sex. Animals were randomly assigned to five treatments (8 replicates, 3 piglets/pen): negative control (NC, basal diet), positive control (PC, basal diet with 120 mg/kg chlorohydroxyquinoline), and the basal diet supplemented with the additive at 200, 400, and 800 mg/kg (NC200, NC400, and NC800, respectively). Piglets were weighed on days 1, 7, 14, 28, and 43 to determine body weight, average daily gain, average daily feed intake, and feed conversion ratio. On day 41, fecal samples were collected from piglets with body weights closest to the block average (8 animals per treatment; $n = 40$) to determine counts of total and thermotolerant Enterobacteriaceae and lactic acid bacteria. Blood samples were collected from the same animals to measure interleukin-6 (IL-6) and interleukin-10 (IL-10) concentrations. Data were analyzed via GLM for linear and quadratic effects, using Dunnett's test for PC comparisons. Cytokines were assessed by Kruskal–Wallis and Mann–Whitney tests. Significance was set at $p < 0.05$, with trends at $0.05 \leq p \leq 0.10$, using SPSS software. Results showed that additive levels were comparable to the PC in terms of growth performance ($p > 0.05$). Additionally, a positive linear effect was observed for lactic acid bacteria counts as inclusion levels increased ($p = 0.031$), while NC200, NC400, and NC800 tended to have higher counts compared to the PC group ($p = 0.061$). This finding may be related to the ability of the acidifiers to reduce luminal pH, favoring the establishment of beneficial populations. Regarding the inflammatory response, the two highest additive levels (NC400 and NC800) showed a trend toward reduced IL-6 levels compared to the PC ($p = 0.057$). This result demonstrates synergistic actions of the bioactive additive; besides positively modulating the microbiota, it suggests a potential to attenuate cytokines associated with increased intestinal permeability and bacterial translocation. In conclusion, the microencapsulated bioactive additive performs similarly to the AGP regarding growth performance, while exhibiting superior effects on gut health and inflammatory response by stimulating lactic acid bacteria proliferation and potentially reducing pro-inflammatory cytokines.

Late lactation represents the main time-window for sow-to-piglet transmission of persistent gut strains

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The gut microbiota plays a key role in the health of piglets. Microbiota transmission from mother to offspring could be a relevant leverage to shape microbiota of piglets for preventing post-weaning digestive disorders. This study aims to better characterize the mother-to-offspring microbiota transmission and persistence by using a metabarcoding approach based on long-read sequencing of the 16S-ITS-23S region. Fecal samples were collected from 17 families, each including one sow and three piglets, sampled at different stages: 110 days of gestation, early (d6) and late lactation (d28) for the sow; early lactation (d6), late lactation (d28), and 5-day post-weaning for the piglets (n = 204 samples). Amplicons were generated following the PacBio Barcoded Overhang Adapters protocol. Sequencing was performed on a PacBio Sequel II system using HiFi long-read technology. Reads (n = 11,550,265) were analyzed using the FROGS 5.1 pipeline for long reads. To achieve near-strain-level resolution and analyze the temporal dynamics and vertical transmission of the microbiota, a putative strain (PS) reconstruction method was developed to group ASVs (Amplicon Sequence Variants, n = 6064) affiliated with the same bacterial species based on their covariance across the whole dataset ($r \geq 0.9$, n = 4857 PS). As expected, the diversity of the piglet microbiota, initially distinct, converged toward that of the sows by the end of lactation with a progressive diversification during lactation. Within individual sows, 27 ± 6% of the PS were persistent from late gestation until the end of lactation. In piglets, only 4.2 ± 2.5 % of PS were detected both at d6 and d28 and remained present until 5 days after weaning. Persistent PS in piglets were affiliated predominantly to Limosilactobacillus reuteri, Lactobacillus amylovorus, Holdemanella porci, Bifidobacterium pseudolongum, Limosilactobacillus mucosae, and Romboutsia ilealis. The PS shared between piglets and sows were significantly higher within the same family than between unrelated piglets and sows at all stages ($p < 0.05$), except when piglets were at the end of lactation (d28) and sows were at the end of gestation. The PS shared from the sow to its own piglets was the highest at the end of lactation (35 ± 7% and 29 ± 6% in piglets at d28 of lactation and 5 days after weaning). Finally, 2.7 ± 1.6% and 15.4 ± 5.6% of the PS transmitted by the sow persisted in piglets from day 6 of lactation to 5 days after weaning, and from day 28 of lactation to 5 days after weaning, respectively. For the latter window (day 28 of lactation to 5 days post-weaning), the species most frequently associated with transmitted and persistent PS across all pig families were Sodaliphilus sp004557565 (109 distinct PS), Sphaerochaeta globosa (20 PS), and Cryptobacteroides sp900316045 (40 PS). These results highlight the contribution of maternal transmission to the establishment of the post-weaning piglet gut microbiota and suggest late lactation as a key window for sow-to-piglet microbiota transfer.

Lactobacillus postbiotic in gestating and lactating sows: effects on sow microbiota, colostrum omics composition, litter growth and health

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Nutritional management of the sow during late gestation and lactation plays a pivotal role in promoting both maternal and piglet performance. Targeted nutritional strategies during these critical phases can influence multiple parameters, including shift in the gut microbiota, colostrum composition and overall piglet growth and health. Among these, postbiotics have emerged as a promising intervention in swine. This study evaluated the effects of gestational and lactational supplementation with Lactobacillus postbiotic (heat-inactivated L. fermentum and L. delbrueckii) on sow and piglet performance, colostrum composition, and gut microbiota. Three weeks before farrowing, 210 sows were assigned to a control group (G0; 42 sows) or a postbiotic-supplemented group (G20; 20 g/t; 168 sows). At farrowing, G20 sows were further allocated to four groups (42 sows/group) receiving 0 (G20-L0), 10 (G20-L10), 20 (G20-L20), or 100 (G20-L100) g/t of postbiotic during lactation. Piglets were weighed at birth (d0), d6, d14, d28 (weaning), and d42 (post-weaning), while mortality and exclusion were daily recorded. Growth performance data were analysed using a linear model, including diet, parity order and batch as factors, litter size and initial body weight (BW) as covariates. Postbiotic supplementation during late gestation (G20) increased piglets' birth weight compared to G0 ($p<0.05$). All postbiotic groups had a higher piglets' BW at d6 compared to G0-L0 ($p<0.001$). At d28, G20-L10 had higher piglets BW than G20-L20 ($p<0.05$), but it did not differ from G0-L0. All postbiotic groups had a higher ADG at d0-d6 than G0-L0 ($p<0.001$). Cumulative mortality during lactation was significantly reduced in G20-L0 and G20-L100 compared with G0-L0 ($p<0.01$). Postbiotic supplementation did not affect immunoglobulins in colostrum. Colostrum fatty acid profiling showed no significant differences between gestation groups. However, a tendency toward a lower HPUFA n-6/n-3 was observed in G20 ($p=0.05$). Multivariate analysis (sPLS-DA) of colostrum miRNAs identified miR-28-5p as discriminative (PC1=0.78) for the G20 group. Metabolomic analysis characterised G20 colostrum with metabolites associated with branched-chain biosynthesis, arginine and proline metabolism, tyrosine metabolism, and glycerophospholipid metabolism. Gestational postbiotic supplementation enhanced sow's faecal microbial richness ($p=0.01$) and beta diversity ($p=0.01$, $R^2=0.06$). Differential abundance analysis highlighted enrichment of Lachnospiraceae, Firmicutes, and Enterococcus faecium in G20, while G0 was enriched in Firmicutes and Bifidobacterium thermophilum. Functional profiling revealed upregulation of pathways related to fermentation and energy metabolism in G20, contrasting with amino acid degradation and thiamine metabolism in G0. In conclusion, postbiotic supplementation in gestation modulated sow gut microbiota and colostrum metabolome, improved early piglet growth, and reduced pre-weaning mortality. The observed microbial and functional shifts suggest enhanced maternal fermentation and energy metabolism, likely involved in changes in colostrum amino acid and lipid pathways relevant for neonatal growth. Overall, these findings support a gut-mammary axis mechanism underlying improved early piglet performance.

Effect of Dietary Zinc Supplementation on Growth Performance and Health of Piglets during the Early Rearing Phase

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Zinc (Zn) plays a crucial role in immune competence, intestinal integrity and growth performance in piglets, particularly during the critical post-weaning period. Historically, pharmacological levels of zinc oxide (up to 1,500 ppm) were widely used to control post-weaning diarrhea, however, environmental concerns have led to a substantial reduction in the authorized inclusion levels (currently 150 ppm) in Europe. However, during the post-weaning period, piglets have a very low intake of feed, and the 150 ppm Zn may not meet their physiological needs. Recent Danish results suggest that newly weaned pigs have a Zn requirement around 1400 ppm for optimal production, blood Zn status and in terms of minimizing the risk of diarrhea. In contrast, 150 ppm Zn may oversupply older pigs relative to their requirement leading to unnecessary Zn excretion to the environment, without negative effects on production and health, which will reduce the Zn. In the present study, we compared assessed the negative effects of a very low level of zinc in the diet, which does not meet the animal's physiological needs, compromises its health, and may lead to additional antibiotic treatments. In this study, seventy-two weaned piglets (28 days of age) were allocated to two dietary treatments: a control diet containing 150 ppm Zn (CT) and a Zn-supplemented diet containing 1,400 ppm Zn from zinc oxide for two weeks post-weaning. Animals were housed in 12 pens per treatment, with three piglets per pen. Feed intake was recorded daily and body weight weekly. Diarrhea incidence was monitored daily using a 0–3 category fecal consistency score. Performance results were analyzed using a variance test with feed as the variance factor; for diarrhea occurrence results, the T-test was used. The JMP pro 17 program was used for statistical analysis. Average daily gain was significantly higher in the Zn-supplemented piglets during the first week (0.33 vs. 0.28 kg/day; $p = 0.0023$), but final body weight was not affected by treatment ($p > 0.05$). Weekly and overall average feed intake per pig did not differ between treatments ($p > 0.05$). Feed conversion ratio was not affected ($p > 0.05$) and the value obtained was 1.53 (1.58 and 1.47 in the CT and Zn-supplemented group, respectively). Zinc supplementation significantly reduced diarrhea incidence ($p < 0.001$), decreasing the number of affected piglets, the duration of clinical signs and their severity. Overall, for the two weeks of trial, the number of piglets experiencing diarrhea was 30 and 6 in the CT versus Zn-supplemented group, respectively. The increased dietary Zn supplementation during the early post-weaning phase improved short-term growth performance and markedly enhanced gut health by reducing diarrhoeal episodes. These findings suggest that strategic higher Zn supplementation right after weaning may support productive and health stability during this critical transition period. Keywords: Zinc supplementation, diarrhoea incidence, growth performance Acknowledgments: This work was supported by the projects UID/00772/2025, DOI https://doi.org/10.54499/UID/00772/2025 funded by the Portuguese Foundation for Science and Technology (FCT).

Effect of increasing the dietary valine-to-lysine ratio on growth performance of average and slow growing pigs during the weaner period

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One fourth of the pig littermates exhibit a slow growth rate from the early weaning phase, resulting in a subset of slow growing (SG) pigs having poorer growth performance during their lifetime than their average growing (AG) counterparts. It has been suggested that increasing the dietary valine (Val) above recommended levels may modulate muscle metabolism leading to improved growth performance. Seventy-two German Large White × Landrace gilt piglets from 18 litters were used in the experiment, with four blocks (farrowing series) needed to complete the experiment. Pigs of average (AG; avg. 235 g/day) and slow (SG; avg. 191 g/day) growth rates were selected based on pre-weaning (day 1-28 of age) growth rates, thus, from each litter two AG and two SG piglets were selected. At weaning (28 days of age) the 36 AG (avg. 8.00 kg BW) and 36 SG (avg. 6.42 kg BW) pigs were, respectively, randomly allocated and evenly distributed (24 pigs per treatment) between three dietary treatments only differing in Val:Lysine-ratio; a control (CON, avg. 7.31 kg BW) with recommended ratio (64%), a treatment with a high ratio (Val-H; 74%, avg. 6.99 kg BW) and a treatment with a very high ratio (Val-VH; 84%, avg. 7.33 kg BW). A three-phase (day 28-35, day 35-49 and day 49-84) feeding regime was applied during the experimental period. Pigs were housed in pens of four animals (two AG and two SG per pen) with ad libitum access to feed and water. The data were analysed by applying ANOVA from the 'nlme' package in R (R Project) using a linear mixed-effects model to estimate BW and average daily gain (ADG). The model included growth category (AG or SG) and dietary treatment (CON, Val-H, or Val-VH) and their interaction as fixed effects, weaning weight as co-variate, and sow and block as random effects. As intended weaning weight differed (7.97 vs. 6.40 kg BW, $P < 0.0001$) between AG and SG pigs. Final body weight (day 84 of age) was greater for AG compared with SG pigs (38.7 vs. 33.5 kg BW; $P < 0.0001$), with similar differences observed at the starting points of the respective phases (days 28, 35, and 49; all $P < 0.0001$). As intended ADG was greater in AG than SG pigs from day 1–28 (235 vs. 191 g/day; $P < 0.0001$). During the second (day 35-49) and last phase (day 49–84) ADG, respectively, tended to be greater and was greater in AG compared with SG pigs (326 vs. 317 g/day; $P = 0.06$ and 640 vs. 601 g/day; $P = 0.02$). Over the entire experimental period (day 28–84) ADG was also greater in AG than SG pigs (525 vs. 443 g/day; $P = 0.027$). The dietary treatment had no effect on final BW or ADG in overall period, but tendencies were observed for ADG during the pre-weaning period (day 1–28) (CON; 216 g/day, Val-H; 201 g/day, Val-VH; 222 g/day, $P = 0.058$) and for BW day 28 (CON; 7.34 kg BW, Val-H; 6.81 kg BW, Val-VH; 7.41 kg BW, $P = 0.05$), had an effect on day 35 (CON; 8.42 kg BW, Val-H; 8.17 kg BW, Val-VH; 8.18 kg BW, $P = 0.0002$), and tendency day 49 (CON; 13.0 kg BW, Val-H; 13.0 kg BW, Val-VH; 13.1 kg BW, $P = 0.06$). No growth category × dietary treatment interaction effects were observed in any of the parameters. In conclusion, the difference in pre-weaning growth rate between AG and SG pigs persisted throughout the weaning period, resulting in greater BW and ADG. Although large numeric differences in final BW and overall ADG were observed between CON and Val-VH SG-pigs (data not shown), no effect of the dietary treatment was found.

Effect of increasing levels of linseed expeller in nursery pig diets containing Carob meal

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The immediate post-weaning period is characterized by reduced feed intake, immature digestive function, and elevated risk of gastrointestinal disturbance, making diet formulation a critical strategy to maintain gut integrity and growth performance. Among those strategies, feeding linseed expeller (LEX, a by-product of oil extraction) and carob meal (CM, from carob pods or germ) can be used as a source of functional compounds such as omega-3 fatty acids, fibers and polyphenols. Those components can increase digesta viscosity and supply substrates for microbial fermentation in the distal gut, potentially affecting colonization resistance and mucosal protection. The objective of this study was to evaluate increasing levels of LEX in diets containing 2% CM on nursery pig growth performance and fecal score during the first 3 weeks post weaning. The study was conducted at the Cargill Animal Nutrition and Health, Global Innovation Center in Velddriel (NL). A total of 288 pigs (23 d; 7.1 ± 0.7 kg BW) were assigned to six treatments (8 pens/treatment; 6 pigs/pen) in a randomized complete block design (block = initial BW; pen = experimental unit). From day 0 to day 21 post weaning, pigs were fed with the following dietary treatments: negative control (NC, 0% carob, 0% linseed), positive control (PC, 2% carob, 0% linseed), or diets with 2% carob and increasing levels of LEX at 1.0, 2.5, 3.5, or 5.0% partially replacing corn and SBM. From day 21 to 39 post weaning all pigs received a common diet without LEX and CM to evaluate any potential carry-over effect. Diets were formulated to provide similar levels of crude protein (21%) and 1.40% SID Lys (NE 2,565–2,587 kcal/kg). Response variables evaluated included ADG, ADFI, G:F, and BW (days 0, 7, 14, 21, 39 post weaning). Fecal consistency was scored at pen level on d 0, 4, 7, 14, 21, 29 on a 4-point scale (from 1 = normal to 4 = watery). Data were analyzed using linear mixed-effects models (lme4 in R; treatment fixed, block random; $\alpha = 0.05$); ordinal mixed models were fitted for scores. During the first week post weaning, pigs fed PC showed greater ADG (0.189 kg, P=0.07) and ADFI (0.189 kg, P=0.09) compared with NC (0.146 kg and 0.155 kg, respectively), and ADG was also greater (+19%, P = 0.08) compared with pigs fed diets containing LEX (0.156 kg). During d0–21, G:F ratio decreased linearly (P=0.01), representing a 3.5% reduction between 1% and 5% of LEX inclusion, while ADG and ADFI did not differ among treatments. During d21–39, ADG (P=0.08) and ADFI (P=0.06) showed quadratic responses to increasing LEX levels, peaking at 2.5% inclusion (0.697 kg and 1.046 kg, respectively) and declining at 5% (0.670 kg and 1.014 kg, respectively). During d0–39, G:F declined linearly in those pigs that received LEX (P=0.03); ADG and ADFI were unaffected. Fecal consistency did not differ; no diarrhea occurred. Given that feed intake during the first week is a key determinant of subsequent piglet growth, early improvements in feed intake with CM suggest that it may stimulate intake and support initial adaptation to weaning. The health status in this trial was high, with low mortality and good fecal scores across treatments. In conclusion, under these high-health conditions, increasing LEX inclusion reduced feed efficiency in a dose-dependent manner and provided no additional benefit beyond CM.

Multi-omics characterization of sow colostrum and faecal microbiota reveals signatures associated with parity, piglet survival and growth

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Colostrum is essential for neonatal piglet survival and growth, yet its bioactive composition and its relationship with parity and early-life outcomes remain poorly defined. This study aims to characterise the colostrum composition of sows with different parity orders and to identify, using a multiomics approach, the main pathways and bioactive compounds that, together with the microbiota can improve the piglet's survival and average daily gain (ADG) during suckling. Colostrum from 20 sows (7 gilts, 7 second-parity, 6 multiparous) was analysed for proximal composition, immunoglobulins, oligosaccharides, fatty acids (FAs; GC-FID) and untargeted metabolomics (UHPLC-HRMS). Piglet mortality and ADG were recorded until d24, and faecal microbiota (d6, d24) was assessed by 16S rRNA sequencing. Multivariate analyses (sPLS-DA, OPLS-DA) and integrative DIABLO models were applied. Parity did not affect colostrum proximal composition or immunoglobulins, but significantly influenced metabolomic (OPLS-DA: $R^2Y = 0.97$; $Q^2 = 0.52$) and FAs profile (22 discriminated FAs). Gilts had higher abundance of branched-chain FAs, several amino acids (AAs) and peptides (e.g., lysine derivatives), suggesting altered metabolic status. Piglet mortality differed by parity ($P < 0.001$), with second-parity sows showing higher losses. Mortality classes at d 6 were discriminated by 177 metabolites and 2 FAs, and 248 metabolites and 21 FAs at d 24. Both at d 6 and d24 a higher abundance of C18:2 8 trans,10cis discriminated for lower mortality (importance = 1 for d 6 and 0.34 for d 24), whereas increased C22:5n6 characterized higher mortality. Metabolomics pathway analysis indicate arginine biosynthesis and alpha-linolenic acid metabolism as key routes linked to survivability both at d6 and d24. Integrative multi-omics analysis associated lower mortality with higher colostrum C18:2 8trans,10cis, C18:1 5trans, salicylic acid, and increased abundance of Holdemania and Roseburia in piglet faeces. For ADG d0–d24, 151 metabolites and 33 FAs discriminated classes. Higher ADG (>240 g/d) was associated with colostrum vitamin E, histidine, branched-chain AAs and specific anteiso FAs (VIP score > 1). Conversely, L-kynurenine and gamma-aminobutyric acid were linked to lower ADG, suggesting maternal inflammatory or stress-related imprinting. Integrated models (accuracy 55–64%) identified combined metabolic–microbial signatures of higher ADG, involving histidine-related metabolites, anteiso FAs and enrichment of gut taxa Lachnospiraceae UCG-009 and Akkermansia. This study confirms the importance of parity order in shaping colostrum composition and identifies several bioactive compounds, some parity-dependent and others parity-independent, that may be associated with improved piglet survival and growth and gut microbiota maturation. These findings support precision nutritional strategies in sows and provide targets for next-generation artificial colostrum supplements for piglets. FundingResearch was supported by BOOSTcolostrum project (P2022Z-TYLS—CUP J53D23018670001) – NextGenerationEU—Piano Nazionale di Ripresa e Resilienza (PNRR) – DD N. 1409 del 14/09/2022.

Semi-targeted metabolomics to explore potential blood biomarkers of oxidative stress in pigs using an E. coli LPS model

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Post-weaning diarrhea caused by enterotoxigenic E. coli remains a major challenge in pig production and is a key driver of antibiotic use. Following the ban on therapeutic zinc oxide and increased antimicrobial restrictions in the EU, new solutions have become essential. Infection with E. coli is associated with systemic inflammation and oxidative stress, which can impair intestinal integrity and exacerbate diarrhea. Biomarker-based approaches may support evaluation of nutritional and management interventions to mitigate oxidative stress, improve post-weaning robustness, and reduce antibiotic use. Lipid metabolites have been associated with immune responses and thus may be potential biomarkers for inflammation as well as oxidative stress. The objective of this study was to explore potential lipid oxidative stress biomarkers via semi-targeted metabolomics using a controlled lipopolysaccharide (LPS) challenge model as a proxy for bacterial-induced systemic inflammation observed during post-weaning enteric disorders. Ten healthy juvenile female pigs (3 months old, 25 ± 1 kg) were selected to ensure uniformity and avoid gender-related effects. Serial blood samples were collected from pre-infusion up to 72 h post-LPS challenge. Two concentrations of LPS (0.75 and 1.50 µg/kg BW) from E. coli O111:B4 were administered. A semi-targeted lipid-focused metabolomics approach was applied to explore potential oxidative stress biomarkers. Data were analyzed using multivariate methods to identify metabolites that discriminated across time independently of the LPS dosage. Cortisol was identified at level 1 with a standard and showed a clear response following LPS infusion ($P < 0.001$), peaking at 2 h post-challenge. Plasma cortisol was strongly correlated with rectal temperature across both LPS doses ($r \approx 0.65$, $P < 0.001$), consistent with its role in the inflammatory response. Linoleic acid and arachidonic acid, and the lipid peroxidation marker 8-iso-$PGF_2\alpha$, were identified at level 1, however, they did not show a clear response to LPS. The absence of a clear 8-iso-$PGF_2\alpha$ response may reflect methodological constraints, as omission of alkaline hydrolysis likely limited quantification to free rather than total F_2-isoprostanes. A serotonin-related metabolite, glutamine and an oxylipin 10-HOME were tentatively identified at level 3. A pronounced response was observed for the serotonin-related metabolite at 3 h post-challenge ($P < 0.01$) and for glutamine at 8 h post-challenge ($P < 0.01$), whereas the oxylipin 10-HOME exhibited a rapid but less distinct response at 0.5 h after LPS infusion ($P < 0.01$). Serotonin has been suggested to influence immune cells, while glutamine may serve as a metabolic substrate for lymphocytes and a modulator of pro-inflammatory cytokines. The rapid increase in 10-HOME may indicate involvement in the early inflammatory response to LPS. However, further research is needed to confirm the identity of the tentative metabolites, clarify their role in inflammation and oxidative stress, and to assess their suitability as potential biomarkers during post-weaning enteric challenges. As pigs are particularly vulnerable during the pre- and post-weaning periods, early application of potential biomarkers may serve as an opportunity to maintain gut resilience and avoid the onset of diarrhea by early nutritional intervention. This work is part of the PIG-PARADIGM project, funded by the Novo Nordisk Foundation (NNFSA210073688).

Effect of tannin of Chestnut and MGM-P on early weaning stage of piglets

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Background In our laboratory has found condensed tannins MGM-P that from Quebracho not only effects on post-weaning diarrhea also improve growth performance of piglets (Ma et al., 2021). The results showed that adding 0.3% concentration of MGM-P demonstrated a better effect in alleviating post-weaning diarrhea than 0.2%. When compere the concentration of MGM-P 0.5% and 1.0% (Ma et al., 2024) found that the 0.5% concentration group had better effects to improve the growth-promoting properties. However, the 1.0% concentration group showed severe anti-nutritional effects, reducing feed conversion efficiency and daily weight gain. Objectives To find the optimal tannin as a feed additive for growth promotion and anti-diarrhea effects and to expect synergistically effect on prevention diarrhea and growth performance the two tannins has conducted. Addition of 0.75% chestnut tannin and combined 0.5% MGM-P and 0.75% chestnut tannin has conducted. Results 1. Growth Performance There are no fatalities occurred during the experimental period. The ADG in 0.75N group was lower than that in the C group. The ADG of the MN group was much lower than that of the C group, but the difference was not statistically significant. Although the ADFI and FCR among the three groups did not show significant differences, the 0.75N and MN groups were showed tendency lower compared to the C group. 2. Blood Hematology Analysis, the RBC levels in both in 0.75N and MN groups at w6 and w7 were significantly lower than those in C group that showed anemia phenomena. 3. The result of WBC levels was significantly lower in 0.75N and MN groups than that in C group at w6. 4. Diarrhea incidence The diarrhea incidence in C group was 41.7%, and 25% in MN group. However, no diarrhea occurred in 0.75N group, significantly lower than that in C group. Discussion & conclusion The present study showed that Chestnut tannin addition level when 0.75% and combined with 0.5% MGM-P showing the tendency negatively affect growth performance. The weekly ADG changes is also showing tendency decrease even if was no significant. The RBC levels from third week, in two tannin-treated group were significantly lower than those of the control group. Ma, Min, et al. (2021,2024) demonstrated that MGM-P even if added from 0.3% to 1.0% there is no anemia phenomena had happened in piglets. Thus, these results might be caused by 0.75% chestnut tannin addition. The WBC showed higher in control group, it might be related with diarrhea, that the incidence of diarrhea in the C group was higher compared to the MN group, and no diarrhea occurred in the 0.75N group, indicating 0.75% chestnut tannin significantly suppresses post-weaning diarrhea. Our study showed Tannins, as a primary component in anti-diarrheal drugs, are known for their astringent and anti-inflammatory properties. This aligns with our findings where the tannin-treated groups, particularly the 0.75% Chestnut group, had dark, harder solid feces, consistent with the fecal moisture measurements showing the lowest moisture content in the 0.75N group. Therefore, the strong anti-diarrheal effect of 0.75% Chestnut tannins in this study is likely due to its astringent properties. The discrepancies between the 0.75% Chestnut results and previous 0.75% MGM-P experiments may be attributed to differences in tannin type, purity, chemical structure, and the timing of creep feed.

The aWISH vision: Improving welfare of pigs and chickens by automated monitoring of animal welfare indicators at slaughter

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aWISH (Animal Welfare Indicators at the Slaughterhouse) is a four-year Horizon Europe Research & Innovation Action (Grant number 101060818) that started in October 2022. The project responded to a call aiming to support policy makers and other actors, such as business operators, in monitoring and improving the welfare of farm animals, with special attention to automated data collection and exploitation at different levels, ranging from individual farms to regional/national and EU-level. The project focuses on automated monitoring of animal-based welfare indicators at the slaughterhouse with the goal to document and ultimately improve the welfare of broiler chickens and fattening pigs during each production phase (farm, transport and slaughter). This approach was developed in close collaboration with all actors involved, from primary producers up to policy makers and citizens through several workshops, interviews and surveys. Now that the project is nearing completion, an overview of the main achievements, lessons learned and way forward is presented. At the heart of the project are six pilots spread across Europe, where a total of sixteen new sensor technologies were developed, tested and validated. Each pilot consists of a slaughterhouse with a local research partner, associated farms and various technology providers. The four pig pilots are located in the Netherlands, Spain, Austria and Serbia, while the two broiler pilots are situated in France and Poland. For pigs, technologies were developed to measure ear lesions, skin lesions, tail length, tail lesions, blood stress parameters, stress vocalisations, tear staining, stunning effectiveness, environmental parameters, body weight, lung lesions and coughs. The broiler technologies document hock burn, footpad lesions, catching lesions, back scratches, stress vocalisations, activity, environmental parameters, heat stress prediction and an app for on-farm welfare self-assessment. These technologies are mainly installed in slaughterhouses, although some are also installed on-farm. For the new sensor systems, TRL levels currently range from 2 to 7, with an average of 6. To support future implementation, an interactive catalogue of both commercially available technologies and research developments for the routine assessment of pig and chicken welfare indicators, covering the five animal welfare domains, has been developed and is updated half-yearly. Furthermore, two methodologies were developed to aggregate welfare indicator outcomes using expert elicitation: an indicator-based aggregation model and a situation-based model. Sensor data complemented by metadata from focal farms and socio-economic and environmental metrics are stored and processed on a custom-made aWISH data platform. The different actors in the production chain receive feedback on relevant individual and aggregated indicator scores through multiple interactive visualisations and dashboards, allowing, for example, time-series monitoring and benchmarking. The interface also provides users with access to best practice guides to address specific animal welfare problems. The platform is further used to document the welfare status at operator and regional/national level, identify risk and success factors, and investigate the effect of the feedback loop on pig and broiler welfare. Finally, the impact of several welfare-improving initiatives on welfare outcomes and socio-economic metrics was assessed. Further expansion and development of the aWISH approach holds promise for cost-effective monitoring and improvement of the welfare of meat-producing livestock throughout Europe.

Monitoring on-farm welfare by using animal-based indicators at slaughter – past and future

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On-farm animal welfare (AW) inspections can be very useful and is a necessary tool for checking the resources and management provided to the animals, with a focus on prevention of AW related problems, and the state of the animals during different phases of rearing. Nevertheless, in-farm inspections can only provide an instant snapshot, and because such inspections are not carried out that frequently – e.g. for reasons related to resources at the competent authorities – there is an obvious risk that AW problems may go undetected. However, most farm animals will sooner or later end up at the slaughterhouse. This means that animal-based indicators reflecting the on-farm conditions can be assessed at the slaughterhouse, and incorporated into the overall AW control system, in addition to on-farm inspections. One such example is the foot-pad dermatitis (FPD) programme for broilers, which was developed in Sweden in the mid-1990s, and has since been implemented – with or without modifications – in several other countries, by legislation or as part of a private standard. The use of food-pad status as an indicator is also mentioned in the EU Broiler Directive. In Sweden, participation in the foot-pad programme, which is part of a larger animal care programme for broilers, is a legal prerequisite for applying higher stocking densities, and this indicator is used for almost all broiler flocks slaughtered in Sweden each year. The foot-pad status is a relevant indicator of the environment and management of the birds, as it is closely linked to the status of the litter material, reflecting factors such as heating and ventilation management, feed composition, hygiene, water quality and litter management. These factors are highly relevant for the welfare of the birds during a production cycle, and in addition to this severe FPD, i.e. the presence of ulcers on the soles of the feet of the birds, is assumed to be a painful condition. A total of 100 feet (one foot per bird, 50 in the beginning of the batch and 50 at the end) are collected from the slaughter line after bleeding and scored by designated slaughterhouse staff under the supervision of the official veterinarian. Each foot given a score of 0 (no lesion), 1 (mild lesion) or 2 (severe lesion), and by weighing these scores in accordance with a pre-set equation, a total flock score between 0 and 200 is achieved. Depending on this score, it can be concluded that everything is fine, or the producer will receive advice within a set advisory programme. In case of repeated flocks with high total scores, the maximum stocking density for the farm in question may be decreased until the problems have been solved. In very severe cases, the competent authorities may be contacted. When the programme was initiated in 1994, the average FPD flock score was 43. In year 2000, the average score had been decreased to 24. Further efforts were made to optimize litter management, feed composition, genetics and other factors, and in 2005, the average flock score was 28, and then as follows: 2010: 34 (median 21), 2015: 21 (median: 11), 2020: 6 (median 0,5) and 2025 (median 0). Some between-year variation is evident, but the general trend, in particular since year 2014, is clearly decreasing. From this, we can learn that it is possible to identify animal-based indicators at slaughter that reflect the on-farm situation in some respects. The next step is to identify indicators that can be assessed automatically, possibly with the help of AI. This still poses some technical challenges, and it is also important to remember that such a system must be regarded by the competent authorities as trustworthy and non-manipulable, to be accepted for replacing a manual system.

An Integrated Welfare Intelligence Platform Across the Animal Production Chain

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Animal welfare indicators are routinely generated at multiple stages of the production chain, including farm management, transport, and slaughter. However, these data are typically fragmented across stakeholders and analysed independently, limiting their capacity to support systemic interpretation. The increasing availability of welfare-related data calls for structured approaches that enable cross-stage exploration while respecting data ownership and confidentiality. Within the framework of the aWISH project, an integrated welfare intelligence platform was developed to serve as the central environment for concentrating, interlinking, and analysing welfare data generated across the production chain. The objective of the platform is to support structured batch-level analysis across production phases and stakeholder groups. The system ingests welfare indicators as recorded in practice. For fattening pigs, examples include environmental parameters (temperature, humidity, CO_2), drinking and feeding behaviour, tail length, lameness, skin/tail/ear lesions, bruises, and pneumonia findings. For broiler chickens, indicators include foot pad dermatitis, hock burns, bruising, dead-on-arrival rates, and human-animal relationship assessments. These heterogeneous datasets are processed and modelled into an analysis-ready structure. Data are interlinked at stakeholder level, batch level, temporally, and based on contextual properties, enabling coherent cross-stage tracking and exploration. Analytical outputs are delivered through interactive dashboards that allow users to monitor batch performance, explore historical trends, and investigate potential associations between management conditions and downstream outcomes. A fine-grained access control mechanism ensures that stakeholders access only data related to their own operations and animals, while enabling comparison against aggregated and anonymised peer benchmarks. In addition, configurable warning mechanisms allow users to define threshold-based conditions and receive alerts when extreme or critical cases are detected. The platform also integrates curated best-practice guidance linked to specific welfare indicators, supporting informed interpretation and management reflection. By bringing together welfare data from different production stages into a single analytical environment, the platform enables stakeholders to examine their results in context rather than in isolation. This integrated perspective supports more consistent interpretation of welfare indicators and encourages coordinated approaches to welfare management across the production chain.

Roadmap for a Prototype Knowledge Platform to Track European Animal Welfare Data.

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Monitoring animal welfare across Europe is a challenge due to the heterogeneous data, the use of multiple indicators for the same welfare concern, and variation in assessment methodologies. To address these challenges, the SOA09 project has developed a prototype Knowledge Platform designed to collect, harmonize, and analyse animal welfare data, providing reliable and comparable information to support policymakers, researchers, and other stakeholders. The platform is being developed following a structured roadmap: first, a network of stakeholders was established to identify requirements, priorities, and available data sources; second, welfare indicators were mapped and harmonized with widely used assessment methods; third, key animal-based "iceberg" indicators were selected to capture multiple welfare outcomes; fourth, standardized data collection templates were created to capture these indicators alongside relevant contextual variables; fifth, templates and indicator selections undergo expert review and pilot validation; sixth, the platform architecture, data management protocols, and governance guidelines were defined; seventh, platform content is developed, and a functional prototype is tested using sample datasets; eighth, user accessibility and role-based permissions are configured; ninth, the platform is launched with training, user manuals, and ongoing support; and finally, a maintenance and update plan is implemented to ensure long-term sustainability. By following this roadmap, the platform aims to enable standardised, high-quality data collection, support and facilitate evidence-based decision-making, and provide a scalable model for future European-wide implementation of animal welfare monitoring.

Experiences and expectations of pig processing plants in the aWISH project to monitor animal welfare using sensor technologies

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The aWISH project is aimed at developing automated systems for monitoring animal-based welfare indicators throughout the pig production chain—from farm through transport to slaughter. As the project approaches completion, this paper provides an overview of the experiences, technological developments, and lessons learned from integrating sensor-, vision-, and AI-based systems in commercial slaughterhouses across Europe. The overarching goal is to enable slaughterhouses to optimize animal welfare, transparency, and product quality by digitally capturing key indicators. Multiple sensor technologies were deployed at partnering farms and slaughterhouses. At farm level, climate sensors continuously measured CO_2, ammonia, indoor and outdoor relative humidity and temperature. Image-based sensors have been used for continuous prediction of liveweight and pig behaviour. Data were visualized in dashboards to support improved farm management. At slaughterhouse level, camera systems and AI algorithms monitored animal handling during unloading and lairage. Sound and motion sensors were piloted as objective welfare indicators. Assessment of effectiveness of stunning process were determined using automatic image collection and use of AI algorithms. Debleeding quality was assessed during exsanguination, complemented by lactate and stress-related blood analyses. An automated tear staining assessment system was implemented in order to test and validate this novel indicator of pig welfare. Ultrasound and fibre-optic probes provided real-time carcass composition data. Newly developed in-line tail-length measurements, including automatic detection of tail damage, were implemented and results fed back to farmers via an online data platform. An automated image-analysis tool was tested and validated for identifying and scoring gross lung lesions associated to respiratory diseases. Integrating sensor and AI technologies demonstrated strong potential for objective, continuous welfare assessment. Preliminary findings indicate that connecting farm-to-slaughter data streams supports predictive modelling and early detection of welfare risks. Automated indicators such as tail damage scoring, environmental measurements, and behavioral data were particularly promising for scalable adoption. Lessons Learned were: (1) Successful implementation requires strong commitment from plant management and IT departments, (2) Reliable on-site IT infrastructure and edge-computing capacity are required for real-time processing, (3) Algorithm training and validation take more time than expected and often require site-specific tuning, (4) Equipment durability must match harsh slaughterhouse cleaning conditions, (5) Integrating data feedback loops to farmers creates new opportunities for chain-wide improvement and for supporting future welfare certification systems. The aWISH project shows that combining sensor, imaging, and AI systems across the pig production chain enables new ways to enhance predictability, welfare assessment, and operational efficiency. Digitalization at slaughterhouses not only supports higher welfare and processing standards but also strengthens transparency and sustainability throughout the livestock chain.

The Eyes & Ears project: automatic on-farm monitoring of animal health and welfare

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Both for indicators of animal welfare and of animal health, monitoring (groups of) animals over time is becoming increasingly important. Modern animal welfare concepts focus more on positive and dynamic aspects of welfare and on supporting the adaptive capacity of animals. Therefore, relevant animal-based measures of health and welfare indicators need to be monitored over time to come to a meaningful assessment at individual or group level. The technical possibilities for automatic monitoring of farm animal health and welfare are developing rapidly. Approaches where vision and/or sound monitoring systems are used hold great promise, as they provide a non-invasive way to monitor health, welfare, specific behaviours and potentially even affective state of farm animals. To date, however, development of practical tools for health and welfare monitoring on commercial farms is still limited. The newly started Eyes & Ears project, an internal project of the European Partnership on Animal Health and Welfare, aims to facilitate the identification, development and validation of such on-farm surveillance tools. The project includes 20 partners from 11 EU countries and will start by reviewing and describing the current technologies that are available for both vision and sound monitoring systems and collecting existing and new datasets. For both vision and sound, we will work on well-defined cases across animal species (including ruminants, pigs, poultry and fish). Here, we also plan to make use of the wealth of approaches and data in some species (for instance: computer vision in cattle) to apply these approaches in other species. In the next step of the project, we plan to develop a dedicated pipeline for analysis of the data coming from the vision and sound monitoring systems, translating the data into relevant information for the farmer. This will be done by applying machine learning techniques. Farmers and other stakeholders will be invited to participate in the decision process about information needed. At this stage, we will focus on a limited number of cases (across species) that are already closer to commercial application. Finally, the project also plans to develop education and training materials that can be used by a wide range of stakeholders interested in developing tools for automatic on-farm monitoring of animal health and welfare. This work was co-funded by the European Union's Horizon Europe Project 101136346 EUPAHW.

Using continuous sensor data on barn climate to evaluate the effect of barn climate advice on pig welfare: a Dutch case-study

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Automated barn climate (BC) monitoring is a widely used tool in precision livestock farming. However, poor sensor maintenance and farmers' hesitation to modify BC settings can result in inadequate air quality for pigs, which has been associated with poor pig health and detrimental behaviour. This highlights the need for expertise in managing BC and the importance of air quality to pig welfare. Therefore, this study aimed to evaluate whether professional BC advice based on continuous barn climate monitoring resulted in improved finisher pig welfare in the Netherlands. Nine pig farms with the Better Life label (1 star) were equipped with the Slimme Stal barn climate sensor (Eng. Intelligent Barn; Connecting Agri & Food) in one finisher unit and followed for two consecutive rounds. In one round, an expert provided BC advice (ADV), whereas this was not the case in the other round (CON). Advice implementation was voluntary (5/9 ADV rounds with uptake of the advice). The rounds were blocked by their starting date (four-week window), and treatment (ADV or CON) was randomly assigned within each block. Pig welfare was assessed twice each round (two weeks after arrival in the finisher barn and in the week before slaughter) using a modified Welfare Quality® protocol for growing pigs. The change in criterion scores and three qualitative behaviour assessment (QBA) PCA component scores between visits were analysed using linear mixed modelling, with treatment as a fixed effect and farm and blocking factors as random effects. Specifically, the criterion scores for Comfort around resting, Thermal comfort, Absence of injuries, Absence of disease, Expression of social behaviours, Expression of other behaviours, and Good human-animal relationship could be analysed, due to proper variation between visits and rounds. Treatment did not affect any of the between-visit changes in criterion scores ($p > 0.05$), nor the first two principal QBA component delta scores representing calm-positive and active-engaged mental states, respectively. The third component, however, showed a tendency for pigs becoming more agitated-distressed during the ADV rounds compared to the CON rounds (LS means [95% CI]: 0.11 [-0.90, 1.11] vs. -0.75 [-1.80, 0.30]; $\Delta = 0.86 \pm 0.34$; $p = 0.054$). In our study, expert BC advice did not improve aggregated pig welfare outcomes. However, effects on non-aggregated welfare indicators are still to be analysed. Further, future analyses will assess whether ADV improved barn climate and how specific air quality parameters relate to (aggregated) pig welfare indicators. This study was part of the aWISH project, which has received funding from the Horizon Europe Research and Innovation Program under grant agreement No. 101060818.

Development of a computer vision system for the assessment of swine welfare from indicators on carcasses.

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The measurement of welfare indicators on pig carcasses offers a cost-effective approach for surveillance of animal welfare on-farm and in pre-slaughter handling. With application of computer vision system (CVS) technologies the evaluation of welfare indicators on carcasses can become continuous, standardized and objective, producing quantifiable data on all animals processed. System development should consider implementation in different abattoir environments, flexibility to integrate with other on-site technologies and be sufficiently comprehensive to provide a strong evaluation of animal welfare from which stakeholders can make informed decisions. This work describes the development of a real-time CVS for the automated assessment of swine carcasses that presently measures four key welfare indicators. For practical installment and operation in a variety of abattoir environments, the CVS uses one, 2D IP camera (H.264 compression) positioned opposite a turn in the conveyer rail after carcasses have received initial dehairing. The CVS was developed with multiple distinct modules that are combined for the total system, with the ability to process on edge hardware within the abattoir, on a cloud system, or a hybrid of the two. A tracking and image acquisition feature identifies individual pigs, the carcass position, captures and stores images of the lateral and dorsal side of each carcass as it passes the camera. Using object detection and neural networks, the CVS divides the carcass into different anatomical regions of relevance to pig welfare and carcass quality assessment; the head and shoulder, mid-section, hindquarters and tail (lateral side), and the back of the pig, tail, left and right ear (dorsal side). Welfare measures are then taken relevant to each anatomical area. Lesion detection modules identify lesions as absent or present, thereafter classifying them into levels of severity; mild, moderate and severe (skin), mild and severe (tail lesions, hernias) and tail length (undocked, or ¾, ½ or ¼ docked tail). The severity of skin and tail lesions is calculated from the ratio of skin surface presenting lesions to the total visible skin surface (per body area), from which the carcass is classified based on the ratio threshold. For tail lesions a secondary colorimetric analysis is applied to characterise red and black (necrotic) tissue, improving the accuracy of assessment. System evaluation was performed using videos of carcasses taken from one abattoir, in which carcasses had been assessed by human observers and classified into severity. Videos were spliced together to provide a range of lesion severity per welfare indicator for the CVS to evaluate (120 carcasses). In the current stage of development, our system achieves correct classification of welfare indicators with accuracies of 93.0% for hernias, 86.3% and 90.4% for dorsal and lateral skin lesions, respectively, and 86.8% for tail lesions. Tail length can be estimated with a magnitude of error of 19.82 px^2 (represented by mean square error) by geometric analysis with an image pixel to cm conversion factor for the tail area. Operating at 30.31 frames per second, this modular framework offers a scalable method for real-time extraction of multiple welfare indicators in industrial settings with the flexibility to add modules. An update on work in progress will be given: the external validation of this CVS in different abattoir environments and development of batch identification for processing data by farm.

Interactive catalogue of sensor technologies for automated welfare assessment at pig slaughterhouses

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Because of increasing public concern for animal welfare, it is necessary to find methods for its assessment that are valid and can be implemented routinely. Welfare assessment should rely mainly on animal-based measures due to their higher validity and relevance compared with environment-based measures. Recent technological advances in sensor technologies enable us to monitor animal-based measures in a continuous and systematic way. Assessing animal welfare at the slaughterhouse through technological implementation allows an objective and continuous evaluation of large numbers of animals. Within the European project aWISH (101060818), a catalogue of sensor technologies was developed to measure welfare indicators in pigs for automated welfare assessment at slaughter. A systematic literature search across three databases identified the most relevant pig welfare indicators, followed by a commercial search to detect technologies currently available or at the prototype stage. Subsequently, 39 experts in technology and animal welfare were consulted to verify and complement the compiled information. As a result, an interactive catalogue comprising 64 technologies was created, of which 14 correspond to prototypes, including computer vision systems (n=38), weighing scales (n=9), flow meters (n=5), acoustic sensors (n=5), thermal (n=5) and infrared cameras (n=2). Technologies are classified according to assessment stage (antemortem, stunning, postmortem), welfare domain (nutrition, environment, health, behaviour or mental state), specific indicator and technology type. Antemortem technologies cover all five welfare domains, whereas stunning and postmortem sensors are restricted to the health domain. During the antemortem phase, tools monitor body weight and water consumption within the nutrition domain; cough within the health domain; tail-biting and activity within the behaviour domain; vocalisations and human–animal interactions within the mental domain; and noise, body temperature and pen space estimation within the environmental domain. Stunning sensors assess stunning effectiveness and killing efficacy, while postmortem technologies evaluate tail and lung lesions. The catalogue is presented as an online interactive tool that enables the selection of the most appropriate technologies for monitoring welfare indicators at the slaughterhouse level and is updated every six months to incorporate emerging technologies.

Ensemble Machine Learning Framework for Automated Cough Detection in Pigs

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The monitoring system for livestock cough detection is essential factor for early warning of respiratory diseases in pig farming and is vital for effective animal welfare and productivity management. Respiratory diseases are one of the most frequently occurring in swine production facilities and may reduce the feed conversion and growth rates, lower pork quality, and increase treatment costs. Therefore, detection of coughing, as the most common symptom of respiratory diseases in pigs, is crucial for the early identification of disease onset. However, complex acoustic conditions in such facilities comprising a range of animal sounds such as screams and sneezes that share similar acoustic properties with coughs, make this problem considerably challenging. The aim of the study was to determine the feasibility of enhancing the accuracy of cough classification based on the sound derived from microphones installed in two separate pig pens utilizing both conventional machine learning and deep learning approaches. The recordings were made at a sampling rate of 8 kHz and comprised of various environmental sounds, including animal vocalization such as coughing, screaming, sneezing and eating. To address the cough classification problem, two distinct approaches were employed in this study: the first utilized time-domain acoustic features with a Support Vector Machine (SVM) algorithm, while the second leveraged frequency-domain features in the form of spectrograms with a ResNet-18 model. The first approach relies on domain knowledge to extract features known to be effective for cough detection, whereas deep learning approach allows features to be learned automatically, eliminating the burden of feature engineering process. Initially, each audio signal was pre-processed and 60 second intervals containing at least one coughing instance were selected for further analyses. Each interval was then divided into 1-second windows and further represented with a total of 21 acoustic features followed with backward feature elimination process and SVM algorithm to identify the most discriminative set for cough classification task. Alternatively, the 1-second windows were transformed into spectrograms and used as input to a neural network model. Instead of using discrete class predictions, the class probabilities from each window produced by the individual models were combined, and the final predictions were obtained as a weighted sum of the probability scores from the two approaches. In order to fill the instantaneous gaps in coughing and non-coughing segments, an additional post-processing step was applied using stacked median filters. The window sizes of the filters, as well as class probability weights for each model, and the class discrimination threshold were optimized on the training/validation dataset and subsequently evaluated on the test set. The proposed methodology accurately detected coughing instances, achieving an overall score of 0.78 F1 score on the test set, thus outperforming the individual approaches by 5% compared to the post-processed SVM approach with acoustic features and 7% compared to the post-processed predictions of ResNet-18 using spectrograms.

Validation of a Computer Vision–Based Tail-Length Measurement Algorithm for Finishing Pigs at the Slaughterhouse

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Tail-biting in fattening pigs is a multifactorial behavioural disorder caused by an interaction of internal and external risk factors. A common procedure to reduce the risk of tail-biting is tail-docking. Routine tail docking is prohibited in the EU; however, it can be assumed that a high percentage of finishing pigs are still tail-docked. The risk of injuries from tail-biting is higher in pigs with intact tails. Hence, tail length, along with tail lesions can be considered as iceberg indicators when assessing animal welfare, but objective, cost-efficient, large-scale on-farm monitoring is currently not feasible. Therefore, a computer vision-based algorithm to measure tail length at the slaughter chain was developed and validated in a slaughterhouse in Austria. A stereo 3D camera setup with pattern projection and auxiliary illumination, capturing five images per carcass, was installed on the slaughter line upstream of the decontamination area. To enable individual animal recognition, an OCR algorithm was applied to identify individual slaughter numbers on both lateral sides of the carcass. Tail length was measured using a two-step approach. First, tails were segmented from the carcass using a retrained YOLOv8 segmentation model applied to the 3D images. Altogether 1,252 3D images were annotated in CVAT and split into a training (876), validation (188) and test (188) datasets. Precision-recall curve showed high precision maintained across the full recall range; the resulting average precision of all classes yielded mAP@0.5 = 0.993. Second, geometric length estimation was performed by applying a classical image-processing pipeline to the predicted tail masks. To evaluate the developed tail length algorithm, 1,102 tails were manually measured on the slaughter line by means of a 'TailSizer' and were defined as the reference data. For statistical analysis, 688 measurements remained. The average measured tail length was 20.3 ± 2.56 cm, ranging from 2.00 to 40.0 cm, and the computer-based tail length 20.3 ± 7.12 cm, ranging from 2.10 to 39.2 cm, respectively. A simple linear regression model ($R^2 = 0.991$, $p < 0.001$) was fitted, showing a strong linear association between computer-based algorithm and measured tail length. Spearman rank correlation coefficient was 0.955 ($p < 0.001$), and the RMSE 2.13 cm. Results demonstrate a small systematic difference between the tail length algorithm and the reference data. Implementing a computer vision–based tail length measurement system at the slaughterhouse can support improvements in on-farm animal health and welfare while providing objective metrics for monitoring legal compliance and enabling welfare-based subsidy schemes in the pig industry.

Automated real-time monitoring of corneal reflex using computer vision to assess stunning effectiveness in a commercial pig slaughterhouse

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Ensuring effective stunning during slaughter is an ethical, legal, and social requirement in livestock production. In commercial slaughterhouses, the assessment of consciousness is largely based on evaluations performed by trained operators. Specifically, in pigs, the corneal reflex is widely used due to its sensitivity and because it is the first reflex to appear when an animal regains consciousness. However, its evaluation remains labour-intensive, which limits its feasibility for continuous assessment. As a potential solution, the use of technologies in slaughterhouses is gaining more interest, as it enables individual, continuous, real-time and objective assessments. This study presents the results of a field implementation program of an artificial intelligence-based system designed to automatically detect the corneal reflex in pigs stunned with CO_2 under commercial conditions. The system combines a high-resolution industrial camera and an air stimulus directed towards the cornea. A synchronised control unit triggers the stimulus and video capture, while a neural network processes the recorded sequences to detect blink responses in real time. The algorithm operates under the premise that a blink is detected by a change in eye morphology, monitoring the eye throughout the entire sequence and classifying it as open or closed. The sensor provides on-site alerts to enable immediate corrective action if required. The system was developed over multiple operational days in a commercial slaughterhouse processing 550 pigs per hour, generating more than 16,000 automated corneal reflex assessments. Some positive detections were recorded, although the great majority were associated with optical artefacts arising from operational conditions, such as movement, water, or dirt. However, one positive case was confirmed by expert observers, demonstrating the practical capability of the system to identify potential blinks. The expanding dataset is currently being used to refine detection robustness, particularly in face and eye localisation under challenging lighting and movement conditions. Beyond technical development, this work contributes to the discussion and validation of air stimulation as an alternative to traditional manual corneal testing in high-throughput environments. The results show the potential of AI-based systems to support continuous, objective and automated monitoring of stunning effectiveness, representing a step towards more transparent and data-driven animal welfare auditing in commercial slaughterhouses.

Time of last breath inside the stunner as an accurate proxy to necessary durations of exposure in controlled atmosphere stunning of pigs

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Controlled atmosphere stunning (CAS) using high concentration of carbon dioxide (CO2) is the most common method of stunning pigs at the time of slaughter in Europe. A key parameter of these systems according to the Regulation (EC) No. 1099/2009 is the duration of exposure of the animals to the atmosphere, which must be chosen to ensure that the vast majority of animals do not require re-stunning. As part of the project for Testing Inert Gases in order to Establish Replacements for high concentration CO2 stunning for pigs at the time of slaughter (TIGER), experiments were conducted in a commercial Dip-Lift system using a new gassing system, which allowed for residual oxygen concentrations <1%. In total, 1300 pigs were stunned and slaughtered using argon or nitrogen-argon based mixtures with 0% to 30% CO2 or high concentrations of CO2. The necessary duration of exposure was systematically estimated for each of the 9 gas mixtures. This was achieved by dynamically adjusting the duration of exposure for the next stunning sequence based on the evaluation of stunning effectiveness of the preceding sequences. The data was analyzed using a probit model with the gas mixture, residual oxygen and experimental phase as variables. The necessary durations of exposure, along with their confidence intervals were then estimated to ensure that only a predefined proportion of animals require re-stunning. Here we explore the possibility of estimating the necessary duration of exposure statistically using the times of last breathing activity inside the gondola, as an alternative to dynamically adjusting exposure times. For the different gas mixtures, means and standard deviations of the last (breathing) activity were estimated, based on manual video analysis of the stunning progress of all individual animals. To avoid confounding effects of the varying exposure duration in the dataset, methods from the survival analysis (R package survival) were employed to censor data for all animals for which breathing activity was detected after ejection. The data was analyzed using a linear model with the same variables as above. Necessary durations of exposure were estimated from the model as the duration where a set proportion of animals would statistically not take a breath after removal from the controlled stunning atmosphere. Confidence intervals of the necessary durations of exposure derived from this new method lay inside those calculated from the dynamic exposure duration approach for all 9 gas mixtures, with a mean absolute difference of the estimates of 15 s (6.3%). The mean size of confidence intervals deceased from 82 s for those from the dynamic exposure duration to 16 s for the new method. We conclude that necessary duration of exposure can be estimated with high statistical precision based on the time of last breath or overall activity inside the stunner. We propose that the last (breathing) activity may thus be a useful tool to estimate necessary durations of exposure for scientific evaluations of new gas atmospheres.By automating the process of determining the last (breathing) activity in each stunning sequence using computer vision methods, this approach could also be applied under commercial conditions to optimize exposure times for specific animal groups and to monitor deviations from normal operation, enabling fast or automatic parameter adjustments when necessary. Finally, extending this method may also allow identifying individual animals at risk of needing re-stunning after ejection before the need arises.

Detection before occurrence: Predicting stunning effectiveness and re-stunning necessity in pigs via computer vision-based tools

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Effective stunning prior to slaughter is mandatory under Council Regulation (EC) No. 1099/2009 to prevent pain, fear and suffering. Failed pre-slaughter stunning in pigs is a pronounced threat to animal welfare during slaughter. Carbon dioxide (CO_2) stunning is currently the most widespread stunning method for pigs. Animals are stunned in groups within pen-shaped stunners (gondolas) by exposure to high concentrations of CO_2 for a defined duration. Due to stunner design and occupational safety limitations, direct observation of the animals during stunning is limited to video surveillance. Automated detection of parameters related to animal behavior and stunning progress could be used to continuously monitor the animals and detect potential markers for animal welfare issues or risk factors for requiring backup stunning. This study implements a computer vision–based detection of movement of pigs during CO_2 stunning. The initial focus is on determining the time of cessation of movement, which could serve as an early proxy for stunning effectiveness and potentially predict the necessity of re-stunning. Dataset used for training and testing was initially collected within the TIGER (Testing Inert Gases in order to Establish Replacements for high concentration CO2 stunning for pigs at the time of slaughter) project. The data includes footage from the front and back perspectives in the gondola for 1300 pigs (two pigs per gondola), along with manual annotations of movement events and the post-ejection re-stunning necessity for each animal. A pig instance segmentation model using YOLOv8n-seg model by ultralytics [v. 8.4.14] was retrained and validated using 100 manually annotated [80 train, 20 validation] frames extracted from video footage [Label Studio (HumanSignal) + Segment Anything Model, instance brush annotation], achieving preliminary box mAP50-95 of 0.87 and mask mAP50-95 of 0.84, enabling reliable separation and identification of animals despite dynamic background conditions. Subsequently, the trained model was used to segment pigs in video sequences. The movement was quantified using Farnebäck's approach to optical flow for every second frame at 50 fps and the Euclidean length of the flow velocity vector as the flow magnitude. The animals' movement intensity was determined as the mean flow magnitude within the regions of interest determined by the pig instance segmentation model. The raw movement intensity was smoothed with a rolling median (window = 25 frames). Analysis was limited to the stationary period of the gondola, excluding videos with the last movement at other times. The last movement within this period was detected by using the Q3+k*IQR threshold (upper quartile Q3, interquartile range IQR, Tukey's factor k=5). Currently the proposed method detects last movement accurately (absolute error<2.5 s) in 76% of cases compared to manual annotations. Next development steps include detecting the start of stunning sequences and ejection from the gondola and extending analysis up to time of ejection. Future development could focus on the detection of the time of loss of posture and distinguishing between the individual movement types. The finished system comprising the computer-vision based tool, cameras in the gondola and a warning system for the operators could be used to assist scientific investigations of controlled atmosphere stunning systems. In the future, the system could potentially be implemented at commercial slaughterhouses to monitor stunning progress and detect deviations from typical operation and adjust stunning parameters. This work was co-funded by the EU's Horizon Europe project 101136346 EUPAHW.

Validation of Best Practices to reduce tail, skin and lung lesions in pigs based on a survey with animal welfare experts

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As part of the EU Horizon project "aWISH" (Animal Welfare Indicators at the Slaughterhouse), "Best Practice Guides" are developed to improve animal welfare in pig farming, on transport and at the slaughterhouse. To ensure good effectiveness of the recommended Best Practices (BP), scientific experts for pig welfare were consulted in an online survey and asked to evaluate the proposed BP. A literature research was conducted to identify BP to reduce the occurrence of tail/ear lesions (TL), skin lesions (SL) and lung lesions (LL) in fattening pigs. Scientific experts were then surveyed about the identified BP using an online questionnaire. The experts were external scientists from different countries, the aWISH partners and the project's expert panel. Participants were asked to rate the proposed BP on a scale from 0% (unsuitable) to 100% (very suitable). Data were analysed using a mixed linear model with the BP as fixed effect and the expert-ID as random effect. A list of BP, ranked according to their rating result was compiled to include them in the Best Practice Guides. Overall, 47 completed questionnaires were collected. The top BP identified for TL were "Good health status" (78.9% agreement), "Good barn climate" (78.0%), and "Provision of straw as bedding" (70.6%) (all $p = 1.000$). To prevent TL, "organic manipulable material" was favoured compared to "non-organic material" (68.2% vs. 43.7%, $p<0.001$). Concerning SL, "Keeping littermates together from birth to finishing period" received high agreement (81.9%), as well as "Little regrouping/mixing of animals on farm and at the slaughterhouse" (82.7% and 75.3%) ($p = 1.000$). "Manipulable material on farm" was considered less effective for SL (53.9%, $p<0.001$). For LL, "Good barn climate" (88.9%) and "Good hygiene" (83.1%) were the top BP. Keeping pigs on fully slatted floor was not considered an adequate way to reduce LL (34.6%, $p<0.001$). Some BP were identified as effective for several welfare indicators, e.g. "No storage of manure in the stable, under the slatted floor" (TL 53.9% vs. LL 63.6%, $p=0.090$) or "Good barn climate" (TL 78% vs. LL 88.9%, $p=1.000$). "Low stocking density (i. e. more space than EU regulation)" showed similar agreement for all indicators (TL 67.2% vs. SL 68.3% vs. LL 63.8%, $p>0.05$). In this study, a total of 21 BP were identified and validated by experts to reduce tail/ear lesions and skin lesions, as well as 10 BP for reducing lung lesions. The BP will be classified based on the project's results and will be made available online to pig farmers, advisors, and veterinarians in easy-to-read infographics and comprehensive documents.

Integrating Welfare and Economic Assessment of the Weight-Detect AI System in Commercial Pig Production

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Weight sensors have become indispensable in contemporary livestock production, facilitating systematic, data-driven monitoring of animal growth. By eliminating the necessity for manual weighing, these systems not only enhance operational efficiency but also improve animal welfare by reducing handling stress and promoting more sustainable resource utilization (Hasan et al., 2024; Caja et al., 2016). Within the realm of precision livestock farming, automated weight monitoring serves as a pivotal connection between productivity, profitability, and farm resilience, especially when integrated with real-time analytics and AI-based decision-support systems (Losacco et al., 2025). In the framework of the EU-funded aWISH project, an AI-driven weight sensor system – Weight-Detect (WD; PLF AgriTech, Brisbane, QLD) was implemented on a commercial pig farm in Spain (Banhazi et al., 2025). The study aimed to achieve two primary objectives: (i) to assess the system's practical applicability under commercial farm conditions and (ii) to utilize its data outputs to evaluate overall pig welfare. Despite the promising potential of weight-sensing technologies, significant gaps persist in understanding their combined effects on animal welfare and financial performance in real-world farming systems. Addressing this gap is crucial to inform adoption decisions and guide the development of next-generation smart livestock technologies. Consequently, this study concurrently evaluates the welfare and economic impacts of implementing an AI-driven weight monitoring system in pig production. A hybrid data collection methodology was employed for the baseline and sensor-specific data, integrating on-site observations with remote data acquisition. Economic impacts were assessed using the TIPI-CAL model (Chibanda et al., 2020), whereby percentage changes observed on the pilot farm were applied to average Spanish farm models. Five implementation scenarios were simulated to estimate potential economic outcomes. Results demonstrate that early detection of growth stagnation enabled timely intervention during a respiratory disease outbreak, leading to measurable improvements in key "physical" and "mental" welfare indicators. Economic analysis showed that even minor delays in reaching market weight substantially reduced farm income, highlighting the sensitivity of profitability to health-related growth disruptions. However, early interventions facilitated by the WD system reduced treatment costs and performance declines, lowered mortality rates, and protected profit margins. Overall, the findings confirm that AI-driven weight monitoring delivers dual benefits: measurable welfare improvements and tangible economic gains. By enabling earlier, data-informed decision-making, the WD system represents a powerful tool for enhancing both animal well-being and financial sustainability in modern pig production.

Challenges in Establishing Ground Truth for Skin Alterations on Pig Carcasses for Training Computer Vision Algorithms

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Skin and tail lesions are widely used animal-based indicators of welfare in fattening pigs. Extracting welfare-relevant signals that point to problems on farm, during transport, or in the slaughterhouse requires large samples, standardized protocols, and well-trained assessors. Continuous manual scoring is time-consuming, costly, and limited by inter-observer reliability and repeatability. Computer-vision–based algorithms may offer a scalable tool to standardize the assessment of skin alterations (SA). Our preliminary objective was to develop an algorithm capable of detecting any SA; During development, we found that defining ground truth (GT) was considerably more complex than anticipated. Accordingly, the aim of this study was to describe the challenges and complexities involved in defining the GT for SA based on several approaches. A multi-camera system with auxiliary illumination captured five images per carcass was installed on the slaughter line upstream of decontamination in an Austrian slaughterhouse. Initially, 22 SA classes were defined. In total, 886 images were annotated. Inter-rater agreement between two trained veterinary students was evaluated on two datasets (83 and 236 images), each annotated three times. Intersection over Union (IoU) agreement was consistently low across all iterations. Between iterations, assessors were retrained, and several classes were discarded. An object-detection model (YOLOv8m) was trained on a subset of classes with sufficient samples (train 620, validation 133, test 133). The final model achieved a mean Average Precision of 0.252 at IoU 0.5 and 0.107 at IoU 0.5–0.95, indicating moderate detection performance with reduced localization accuracy at stricter overlap thresholds. Mean precision was 0.351 and mean recall (RC) was 0.285. Class-wise performance was heterogeneous: bruises had the highest RC (0.44) but lower precision (P) (0.36) due to many background false positives; old scratches (P 0.39; RC 0.26) and tail lesions (P 0.53; RC 0.31) were moderate; fresh scratches performed poorly (P 0.11; RC 0.03). Error analysis revealed two dominant failure modes: (1) true lesions missed and classified as background and (2) background misclassified as bruises. Detection performance varied substantially across lesion categories and was lowest for underrepresented classes. We therefore adopted an alternative scheme, based on the Welfare Quality® protocol for pigs, collapsing labels to two classes: animal-welfare-relevant vs. non–animal-welfare–relevant. One expert annotated 2877 images across five camera perspectives; 1000 images were used for inter-rater evaluation, yielding Cohen's kappa of 0.52. Image-classification models (YOLOv8) trained on these labels achieved higher accuracies (79%–96%), depending on perspective. However, this discrete scheme ignores the continuous spectrum from non– to clearly welfare-relevant findings. The study identified several challenges in GT: SA exhibited high variability in size, age, severity, and etiology; moreover, the image datasets contained a high proportion of process-induced post- mortem SA, implying that algorithms must be adapted for different slaughterhouses. Consequently, the overall strategy for establishing GT for SA requires revision. Objectively measurable a priori indicators should be prioritized (e.g. electro prods, multiple tattooing, SA attributable to aggressive interactions). In addition, validation should be expanded to encompass ante-mortem assessments in lairage areas, during transport and on farm.

Thermal Stress Detection in Poultry Using Audio Signal Analysis and Supervised Latent Space Structuring

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Thermal stress represents a major challenge for animal welfare and productivity in poultry production systems, particularly in confined slaughter environments. In this communication, we propose a non-invasive acoustic monitoring approach for detecting and characterizing thermal stress under real industrial conditions, jointly analyzed with environmental hygrometric measurements. Microphones were deployed in pre-slaughter holding areas to capture audio recordings of poultry batches, while ambient temperature and humidity were continuously monitored to characterize the thermal load conditions. An initial dataset of few hundreds of audio segments was annotated by domain experts using a dual labeling scheme: (i) emotional valence (positive / negative) and (ii) vocalization type (distress call, contact call, comfort vocalization, etc.). Due to the limited size of the expert-labeled dataset, we developed a semi-supervised expansion strategy based on time-frequency representations and frequency patterns learned from annotated samples to retrieve and label acoustically similar patches in unlabeled recordings from the same production lot. The enriched dataset was subsequently used to train a convolutional autoencoder with a contrastively regularized latent space structured according to both vocalization class and emotional valence. This latent organization promotes explicit clustering of stress-related acoustic states while improving inter-class separability and robustness to environmental noise. To validate the physiological relevance of the learned representations, the latent stress indicators were further analyzed with respect to hygrometric variations, enabling the identification of correlations between acoustic signatures and environmental thermal conditions. The resulting system enables continuous temporal monitoring of acoustic stress indicators and paves the way for deployable, real-time animal welfare monitoring solutions in industrial poultry environments.

Validation of a computer vision model for automated tear staining assessment in pigs

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The extent of tear staining is considered a promising indicator of pig welfare. Yet current scoring protocols primarily rely on human visual interpretation of the stain-to-eye ratio, which may lead to limited inter- and intra-observer reliability due to the inherent visual ambiguity of the task. As a result, this limitation raises concerns regarding the broader reliability of tear staining as a useful welfare measure and highlights the need for a more reproducible assessment method. This study aims to validate a computer vision approach for tear staining detection by comparing its performance with two groups of human annotators with different levels of experience (expert and task-trained assessors). A dataset of 1,000 video-recorded pigs was manually collected at the bleeding lines of two commercial slaughterhouses, one in Spain and one in the Netherlands, with each clip capturing both sides of the pig's face under standardized lighting and at a fixed distance. The computer vision model processes these videos using two deep-learning modules: a detection model (YOLOv11) that identifies suitable frames and a segmentation model (DynUNet) that delineates the tear stain and eye regions, enabling the estimation of staining severity. To compare human and algorithmic assessment, two complementary tear staining assessment protocols are used: an adapted tagged visual scoring scale (TVAS) applied at clip level and manual annotations applied at frame level. Inter- and intra-assessor agreement is quantified using intraclass correlation coefficients for the visual scale and overlap-based metrics (e.g., Dice score) for frame-level annotations. Preliminary results indicate that the computer vision algorithm currently achieves a Dice score of 68% relative to expert annotations, with the full validation study still underway. By systematically comparing algorithmic and human assessments, this study aims to provide an initial validation of automated tear staining detection as a reproducible monitoring tool under commercial slaughterhouse conditions.

An investigation into the effectiveness of an alternative one-step head to body electrocution technique using mains voltage and off-the-shelf electrodes for euthanasia of large pigs (>130kg)

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Pig farmers want user-friendly and cost-effective methods for euthanizing compromised pigs and performing emergency herd culling. Commercially available electrical approaches such as the one-step stun-kill method applies current to the head and chest through specialized tongs. However, the high voltage equipment is costly and must be ordered from select companies. Canada's swine industry is interested in an off-the-shelf one-step electrocution technique utilizing accessible self-adhering electrodes (copper clamps) attached to the head and body at blood-rich locations. Current is then delivered from a 120 Volt alternating current (VAC) power socket travelling through the blood to target the brain and the heart. This technique could make euthanasia cheaper and enable personnel to be hands-off during implementation. The technique has been tested in pigs of five to 125kg, however it has not been validated as effective or humane for heavier pigs. This study evaluated whether one-step electrocution using off-the-shelf electrodes supplied with 120VAC induces unconsciousness and kills large pigs (130-340kg). Cull pigs (n = 21) from three weight categories (i:130-180kg, ii:190-240kg, iii: 250-340kg, n=7 pigs/category) were used. For each pig, weight, backfat (mm), and body condition score (1: emaciated to 5: obese) were obtained. The pig was sedated in a plastic-sided pen with a rubber mat floor, then anaesthetized to a light surgical plane while lying laterally. An electrocardiogram (ECG) and electroencephalogram (EEG) were recorded. Blood pressure was measured in two to five pigs/category. For euthanasia, two electrode combinations were tested: i) copper clamps on the skin of the groin (flank) and the upper lip (CFL); ii) a clamp on the flank and on a chain bridle running through the mouth (BCF). Clamps were connected to an electrical transformer plugged into a North American power socket, which applied 120VAC at 60Hz to the pig for 15 seconds (s) and the voltage and amperage (A) output were recorded. Before and after electrocution, palpebral, corneal, and nociceptive reflexes were tested, in addition to assessing heart and brain activity, and respiration. Preliminary results from 20 pigs (CFL: n=11 pigs, BCF: n=9) showed that 85% of pigs were killed with one 15s application of electricity, immediately displaying no reflexes suggestive of consciousness or respiration, and presence of cardiac fibrillation. Amperage through the pig was 0.50 ± 0.11A (mean ± SD) with CFL and 0.64 ± 0.15A with BCF. Three pigs >300kg were given a second 15s application due to body spasming post-electrocution (n=2 boars), and blinking (sow near full gestation). Following the first application of electricity, all pigs lacked a cardiac sinus rhythm and blood pressure flatlined and dropped below life sustaining levels. Full results will be available for the meeting. One-step electrocution with 120VAC applied through copper clamps consistently disrupted heart function and eliminated all signs of consciousness in. In boars and late gestation sows >300kg, sufficient current may not reach the brain and surrounding neural structures which is a concern for ensuring their unconsciousness and needs evaluation.

A protocol for the assessment of (un)consciousness after stunning during slaughter of cattle

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According to the European Regulation (EC) No 1099/2009, stunning of animals prior to exsanguination is mandatory (with limited exception for religious slaughter applied in some member states). The purpose of stunning is to render the animals insensible to stress, pain and suffering during bleeding. In practice, the animals are to be rendered unconscious before neck cutting. After stunning and during bleeding, the food business operator (FBO) must ensure and control that the animals remain unconscious until death supervenes. From an animal welfare point of view, rapid detection and immediate re-stunning of any insufficiently stunned animal is critical to prevent avoidable suffering. A structured, reliable and operational assessment of unconsciousness at slaughterhouses is therefore essential to minimise avoidable suffering and ensure timely corrective action. This study was carried out within the framework of the European Partnership for Animal Health and Welfare (EUPHAW) Set of Activities (SoA) 14, group 3 "Ruminants" and presents the evidence-based monitoring protocol developed to support official controls during the slaughter of cattle with stunning. The protocol is based on a semi-systematic review of recent peer-reviewed literature, integrates EFSA scientific opinions, and defines critical control points from restraint at stunning to confirmation of death. Moreover, the protocol outlines harmonised procedures based on both animal-based measures (ABMs) and resource- and management-based indicators, suitable for on-site inspection and auditing. The protocol covering the assessment of stun quality in cattle after using a mechanical stunning method suggests that the most reliable and feasible indicators of unconsciousness are loss of posture, absence of righting reflex, absence of rhythmic breathing and absence of spontaneous blinking and corneal reflex, although the latter, i.e. eye reflexes, are by some considered less feasible. The presence of spasms or seizures directly after the stun, the absence of muscle tone or the presence of excessive kicking can be used as indicators of unconsciousness but may require a more skilled observer. The post-stun convulsions may make it difficult to evaluate some of the spinal reflexes. The presence of vocalisations is considered a reliable indicator of consciousness, whereas the absence of vocalisations is not. For some indicators, such as gasping, nystagmus or eyeball rotation, presence indicates concern regarding the stun quality, and more indicators must then be evaluated. The protocol emphasises that no single indicator is sufficient alone. A combination of multiple indicators must be applied to confirm successful stunning and that the animal remains unconscious during the entire process until death by loss of blood has occurred. throughout all key stages: immediately after stunning, at sticking, and during bleeding until the animal is dead. The assessment should be carried out by properly trained operators. Further activities will be carried out in the framework of SOA 14 in order to fully validate this protocol and make it ready to be used in the context of slaughterhouses. This work was co-funded by the European Union's Horizon Europe Project 101136346 EUPAHW.

Pilot insights into repeatable monthly animal welfare assessments on dairy farms

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Reliable animal welfare assessments are essential for benchmarking and improving dairy production regarding societal demand and are mandatory due to §11 (8) of German Animal Welfare Act. Due to high personnel costs to measure animal welfare manually, efforts are currently ongoing to automatise the assessment. One challenge is aligning different data frequencies, such as annual or half year animal welfare assessments and daily or hourly sensor data. In order to automatise animal welfare assessments using daily available sensor data, the repeatability and reliability of manual animal welfare assessments must first be investigated. Hypothesis: We hypothesised that monthly assessments of animal welfare provide repeatable and reliable results in a limited time period. Experimental methods: This study is carried out on four commercial dairy farms in the north-east of Germany with varying herd sizes between 650 and 1,600 lactating cows. In all four farms the cows were held in loose housing systems with cubicles. Welfare assessments are carried out monthly over twelve consecutive months using the standardized Welfare Quality® Assessment protocol. According to the protocol, the welfare principle scores for good feeding, good housing, good health and appropriate behaviour were calculated based on 30 measures. Principle scores are rated on a 0 – 100 scale (0 = worst, 100 = best). The overall welfare score is the sum of the four welfare principles. All evaluations were performed by the same trained observer. Data were analysed at farm level. The data presented here derived from the winter period from December 2025 to February 2026. Statistical analyses: To assess differences in animal welfare assessments across this three-month period, a non-parametric Friedman test for repeated measures was performed including the four individual farms as blocks. All analyses were conducted in R (version 4.4.1). Results: According to the Welfare Quality® Assessment protocol, animal welfare was classified as “acceptable” in three farms during the three-month period. In one farm, the animal welfare classification changed from “acceptable” to “enhanced” and then back to “acceptable” due to a change in the assessment of the principle for appropriate behaviour; the principle for good health remained unchanged with low scores. The welfare principle scores for good feeding, good housing, good health and appropriate behaviour varied between 47.9 – 93.0, 37.0 – 47.4, 12.7 – 33.7 and 33.3 – 51.1, respectively. The overall welfare score lay between 144.9 – 206.8. The overall welfare score and the welfare principles showed no differences within the three-month period ($P > 0.05$). Discussion and conclusion: Even if the overall welfare score and the welfare principles at the farm level were repeatable in winter, only minor changes in welfare principles that were around the threshold for animal welfare classification led to an improved animal welfare classification. With regard to the future automatisation of animal welfare, the weighting used to classify animal welfare should be reconsidered. However, analyses will be repeated including the 12 consecutive months until the end of 2026 to align manually and automatically raised data.

A new protocol for the assessment of (un)consciousness during slaughter without stunning

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In different EU countries, religious slaughter without prior stunning is still permitted under Article 4 of Regulation (EC) No 1099/2009, even if often strictly regulated. However, it remains a critical animal welfare issue due to the variable persistence of consciousness after neck cutting. A structured, reliable and operational assessment of unconsciousness at slaughterhouse is therefore essential to minimise avoidable suffering and ensure timely corrective action. This work was carried out within the framework of the European Partnership for Animal Health and Welfare (EUPHAW) Set of Activities (SoA) 14, group 3 "Ruminants" and presents a practical, evidence-based protocol designed to support official controls during the slaughter without stunning of cattle and small ruminants. The protocol integrates EFSA scientific opinions, DIALREL recommendations and recent peer-reviewed literature, defining critical control points from restraint to confirmation of death. It establishes harmonised monitoring procedures based on animal-based measures (ABMs), suitable for on-site inspection and auditing. Three sequential post-cut checkpoints are proposed, relying on the simultaneous evaluation of primary indicators of unconsciousness, particularly respiratory and ocular responses, together with supportive indicators such as posture and vocalisation. Species-specific time thresholds for expected loss of consciousness are incorporated (30–40 s in cattle; 15–25 s in small ruminants), reflecting anatomical and physiological differences relevant for inspection activities. Clear, decision-oriented criteria are defined, including back-up stunning whenever signs of consciousness persist, abnormal bleeding patterns occur, or any doubt exists regarding sensibility or loss of consciousness. The protocol further addresses key elements under official control responsibility, including adequacy of restraint systems, knife specifications, operator competence, documentation requirements and corrective actions. Particular attention is given to the interpretation of transitional "grey-zone" states and to distinguishing voluntary movements from anoxia-related convulsions, reducing the risk of misclassification during inspections. By providing structured checkpoints, validated indicators (including sensitivity, specificity and feasibility where available) and explicit decision rules, this protocol supports consistent enforcement of animal welfare requirements, enhances transparency of official controls and strengthens the role of competent authorities in safeguarding animal welfare during slaughter without stunning. Further activities will be carried out in the framework of SoA 14 in order to fully validate this protocol and make it ready to be used in the context of slaughterhouses. This work was co-funded by the European Union's Horizon Europe Project 101136346 EUPAHW.

Gasping and gagging in pigs after CO2 stunning: indicator of insufficient stunning or part of the dying process?

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One of the most common methods for stunning pigs at slaughter is exposure to high concentrations of carbon dioxide (CO2). The physiological mechanism involves acute hypercapnia and acidification of brain cells, which depresses brain activity and ultimately induces unconsciousness. However, the stunning process presents two key animal welfare challenges: preventing pain and fear during stunning and maintaining unconsciousness throughout bleeding. Failures in stunning can lead to unnecessary animal suffering and compromise worker safety. Animals must therefore be closely monitored after stunning to ensure they remain unconscious until death by exsanguination. This monitoring relies mainly on behavioural indicators such as the absence of corneal reflex, rhythmic breathing, and vocalisations. However, some indicators can be difficult to interpret after loss of posture. In particular, respiratory-related behaviours such as gasping and gagging may be difficult to distinguish from rhythmic breathing in slaughterhouse environments where high line speeds complicate observation. Therefore, the aim of this literature review was to synthesise current scientific knowledge on gasping and gagging after loss of posture in pigs stunned with CO2 to evaluate their relevance as indicators of stunning effectiveness. Although no unified definition of gasping exists, it is most commonly described as rapid intakes of air through a wide-open mouth, sometimes with head movements and airflow sounds. The literature suggests gasping is associated with severe oxygen deprivation and may reflect brainstem-mediated respiratory reflexes during hypoxia. These movements may occur even when cortical brain activity is markedly depressed, indicating that gasping does not necessarily reflect consciousness but a physiological response to hypoxia. However, its interpretation remains debated: some studies describe gasping as residual brainstem activity or possible recovery of brain function, whereas others interpret it as agonal respiration during the dying process. Gasping has also been reported together with other indicators of insufficient stunning, such as a positive corneal reflex, suggesting the animal may not be fully unconscious. This highlights the difficulty of using gasping as a reliable indicator when assessing stun quality in pigs. Scientific studies on gagging are limited. The behaviour is sometimes described as retching- or choking-like movements but is rarely reported in studies of CO2 stunning under commercial conditions. When mentioned, it often describes behaviours associated with loss of consciousness during CO2 exposure in experimental settings. Consequently, gagging lacks a clear operational definition after loss of posture. In addition, inconsistent terminology raises the possibility that behaviours described as gagging may correspond to gasping, which further complicates interpretation of the literature. In conclusion, this review highlights inconsistencies in how gasping and gagging are defined in the literature. These inconsistencies may create uncertainty for slaughterhouse personnel who rely on behavioural indicators to detect signs of consciousness after stunning. Further research combining behavioural observations with neurophysiological measurements of brain activity is needed to clarify the physiological basis and welfare relevance of these behaviours.

Effect of dietary inclusion of a Gracilaria chilensis by-product on post-weaning growth and health of pigs

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Gracilaria chilensis, a red macroalga, contains phenolic compounds, proteins, lipids and minerals. Following agar extraction from Gracilaria chilensis, the residue underwent micro-filtration (to obtain insoluble fibre and protein) and cross flow-filtration (to obtain soluble fibre and peptides) to produce a Gracilaria by-product. This by-product may have potential as an alternative feed ingredient in pig diets. Our study evaluated the effect of dietary inclusion of this Gracilaria chilensis by-product in post-weaning (PW) diets on piglet growth, whole blood haematology and small intestinal health. At weaning (29±0.96 days; 8.4±1.01 kg), 72 pigs were formed into 10 pen groups containing 3 pigs, and 3 pen groups containing 2 pigs. Pen groups were blocked on pen group size at weaning, sex (entire male and female) and weaning weight. Within block pen groups were randomly assigned to one of two dietary treatments (n=13 pens/treatment): 1. Control diet; 2. Control plus 1% Gracilaria by-product (Gracilaria). Pigs were fed their experimental diets from weaning until day (d)21 PW, after which a common weaner diet was provided until d44 PW. Individual pig weights were recorded at weaning (d0) and d8, 14, 21, 27 and 44 PW. Feed disappearance was recorded for the intervals between pig weighings. At d8 PW, one pig from each pen containing 3 pigs (n=10 pigs/treatment) was euthanised to collect whole blood for haematology and intestinal tissue (duodenum, jejunum, and ileum) for mRNA analysis. Data were tested for normality using the Univariate procedure. Pig growth was analysed using PROC MIXED with day as the repeated measure; haematology and mRNA data were analysed using PROC GLIMMIX in SAS. Dietary treatment was included as a fixed effect, block as a random effect and weaning weight as a covariate when significant in the model. Pen was the experimental unit for growth performance, while individual pig was the experimental unit for haematological and mRNA data. Dietary treatment did not affect body weight, average daily feed intake, average daily gain or feed conversion efficiency at any period up to d44 PW. There was a tendency for mean corpuscular haemoglobin concentration (P=0.06) and basophil percentage (P=0.09) to be higher in Gracilaria pigs compared to Control, while the monocyte percentage tended to be lower in Gracilaria compared to Control (P=0.07). Although the basophil and monocyte ranges were within normal ranges for weaned pigs, this may indicate an altered white blood response, warranting further investigation with, perhaps, a longer intervention period. In the duodenal mucosa, CLAUD4 mRNA levels were lower in pigs receiving the Gracilaria diet compared to Control (P<0.001), suggesting reduced tight junction sealing and increased paracellular permeability in the duodenum in response to Gracilaria. In the jejunum, MUC2 levels were lower in Gracilaria pigs compared to Control (P=0.03), which may suggest a shift in barrier defence with relatively less mucus-based protection and greater reliance on tight junction regulation. In the ileal mucosa, CLAUD4 levels were higher in the Gracilaria group compared to Control (P=0.04), indicating tightening of the distal small intestinal barrier consistent with compensatory reinforcement following upstream permeability changes. Collectively, these results indicate localised intestinal responses to Gracilaria supplementation without negative effects on pig growth performance.

Protein and fiber source and inclusion level modulate feed preference in weaned piglets.

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Weaning is a critical phase in piglet development during which feed acceptance plays a key role in intake and post-weaning adaptation. Two studies were conducted to evaluate the effect of inclusion level on feed preferences of weaned piglets ([Large White x Landrace] x Pietrain; 13 d post-weaning) for diets with different proteins and fiber sources (Trial 1), and of types of processing of fiber sources (Trial 2) using a two-choice preference test adapted from Solà-Oriol et al. (2009). In Trial 1, 144 piglets were allocated to 36 pens (4 piglets per pen), and they were offered double-choice combinations of 3 diets differing in the inclusion level (low, medium, high) of spray dried plasma (SDP) and soy protein concentrate (SPC), as animal or plant protein sources; and sugar beet pulp (SBP) and soybean hulls (SBH), as soluble or insoluble fiber sources. Choices were offered in four different periods of 3 days, one for each ingredient. Piglets showed significant increases in preference ($P < 0.01$) in response to the increasing inclusion levels of SDP from 0 to 3% (28 vs 72%), 3 to 6% (26 vs 74%) and 0 to 6% (27 vs 73%). Preferences for SPC did not follow a clear inclusion dose pattern as piglets showed no significant differences between 0 and 4% of inclusion (61 vs 39%), a decrease in preference from 4 to 8% (65 vs 35%; $P < 0.01$), and an increase in preference from 0 to 8% (37 vs 63%; $P < 0.01$). Increasing SBP levels from 0 to 4% (74 vs 26%) or 0 to 8% (90 vs 10%) significantly decreased feed preference ($P < 0.01$), while no significant differences were observed between 4 and 8% of inclusion (47 vs 43% preference). Similarly, increasing SBH levels from 0 to 2.5% (71 vs 29%) or 0 to 5% (83 vs 17%) significantly decreased feed preference ($P < 0.01$), with no difference between 2.5 and 5% of inclusion (58 vs 42% preference). In Trial 2, 108 piglets were allocated to 36 pens (3 piglets per pen) to assess whether processing methods could improve acceptance of fiber in diets. SBP and SBH were ground at either 3.0 (control) or 0.5 mm, or ground at 0.5 mm and extruded, before being added into the test diets at 8 or 5%, respectively, and compared against a control diet without added fiber. For SBH, it is not clear whether processing mitigated the negative effect of fiber inclusion, as no significant preference differences were found for any of the comparisons: 0 to 5% (59 vs 41%); 0 to 5% at 0.5mm (53 vs 47%); and 0 to 5% at 0.5mm and extruded (46 vs 54%). In contrast, processing of SBP did not improve preference compared to unprocessed SBP as adding SBP significantly reduced feed preference in all cases from 67 to 33% preference ($P < 0.01$). In conclusion, piglet feed preference is strongly influenced by protein source and fiber inclusion level. While it is not clear whether processing strategies can partially overcome the reduced acceptance of SBH, they are ineffective for SBP. These findings emphasize the need to consider both ingredient composition and processing when formulating diets for newly weaned piglets.

Effect of dietary inclusion of a Laminaria hyperborea by-product on post-weaning growth and intestinal health of pigs

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Laminaria hyperborea, a brown macroalga, contains minerals, fibre and fucose-rich polysaccharides such as fucoidan, which is considered a high-value bioactive compound. Fucoidan extraction from Laminaria, through mild acid, alkaline and screw pressing steps produces a Laminaria by-product. This by-product may have potential as an alternative feed ingredient in pig diets. Our study evaluated the effect of dietary inclusion of this by-product in post-weaning (PW) diets on piglet growth, whole blood haematology and small intestinal health. At weaning (29±0.96 days; 8.4±1.01 kg), 72 pigs were formed into 10 pen groups containing 3 pigs, and 3 pen groups containing 2 pigs. Pen groups were blocked on pen group size at weaning, sex (entire male and female) and weaning weight and within block were randomly assigned to one of two dietary treatments (n=13 pens/treatment): 1. Control diet; 2. Control plus 5% Laminaria by-product (LAM). Pigs were fed their experimental diets from weaning until day (d)21 PW, after which a common weaner diet was provided until d44 PW. Individual pig weights were recorded at weaning (d0) and d8, 14, 21, 27 and 44 PW. Feed disappearance was recorded for the intervals between pig weighings. At d8 PW, one pig from each pen containing 3 pigs (n=10 pigs/treatment) was euthanised to collect whole blood for haematology and intestinal mucosal tissue (duodenum, jejunum, and ileum) for mRNA analysis. Data were tested for normality using the Univariate procedure. Pig growth was analysed using PROC MIXED with day as the repeated measure; haematology and mRNA data were analysed using PROC GLIMMIX in SAS. Dietary treatment was the fixed effect, block was the random effect and weaning weight, when significant, was included as a covariate in the model. Pen was the experimental unit for growth performance, while individual pig was the experimental unit for haematological and mRNA data. Dietary treatment did not affect body weight, average daily feed intake, average daily gain or feed conversion efficiency during any period up to d44 PW ($P>0.05$). Regarding haematology, only monocyte percentage was affected by treatment and this was reduced in LAM-fed pigs compared to Control ($P<0.01$). Although monocyte values were within normal ranges for weaned pigs, this finding may indicate an altered white blood cell response to LAM which may warrant further investigation. In the jejunal mucosa, MUC2 mRNA levels were lower in LAM compared to Control ($P<0.001$), suggesting less mucus-based protection in the proximal region of the small intestine. However, in the ileal mucosa, CLAUD4 and MUC2 mRNA levels were higher in LAM compared to Control ($P<0.01$), indicating compensatory activation of both tight-junction and mucus-layer defence in the distal small intestine as a consequence of weakened proximal mucus protection. Collectively, these results indicate some localised intestinal responses to Laminaria by-product inclusion without negative effects on growth performance. Therefore, this macroalgal by-product can be safely incorporated into post-weaning pig diets at dietary inclusion rates of up to 5%.

Effects of pre- and post-weaning zinc and copper supplementation strategies on their homeostasis in plasma, intestinal mucosa and liver in pigs

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The EU restriction on total zinc (Zn) levels in post-weaning diets of pigs cause transient hypozincemia that increases susceptibility to diarrhea. Due to the low post-weaning feed intake, alternatives to in-feed supplementation may help mitigate this issue. This study evaluated the effects of different pre- and post-weaning Zn and copper (Cu) supplementation strategies on their homeostasis in pigs. At d13 of age, 100 suckling pigs were randomly assigned to two oral treatments: S – 2 mL of saline or M – 2 mL of saline with 6.43 and 2.79 mg/L of Zn and Cu as sulfates. At weaning (d21), pigs from both pre-weaning treatments were randomly assigned to two supplementation strategies until d28: W – basal diet devoid of supplemental Zn and Cu and drinking water supplemented with 150 and 65 mg/L of Zn and Cu as sulfates or F – basal diet supplemented with 300 and 130 mg/kg of Zn and Cu as sulfates and drinking water devoid of supplemental Zn and Cu. Between d28-35, all pigs (SW, SF, MW and MF) received a regular diet with 300 and 130 mg/kg as sulfates. Samples were collected at d21, 28 and 35. Statistical analyses used one-way analysis of variance to compare pre-weaning treatments (S vs M) and also each nursery treatment (SW, SF, MW and MF) at d28 and d35 vs pre-weaning values (d21). At d21, M pigs had higher ($P \leq 0.05$) jejunum mucosa, liver and plasma (not for Cu; $P = 0.48$) Zn and Cu concentrations compared to S. At d28, both M groups showed a reduction ($P \leq 0.02$) in plasma Zn compared to d21. In jejunum mucosa and liver, however, Zn concentrations at d28 were not different from d21 in MW ($P \geq 0.66$) but were lower in MF pigs ($P \leq 0.05$). Compared to d21, SF pigs decreased ($P \leq 0.08$) Zn concentrations in plasma and liver but increased in jejunum mucosa whereas SW pigs enhanced ($P < 0.05$) Zn concentration in jejunum mucosa and liver at d28. Plasma (MW and MF) and liver (only MW) Cu concentrations dropped ($P \leq 0.03$) at d28 compared to d21. Plasma and liver Cu concentrations decrease ($P \leq 0.04$) in SW pigs at d28 but increased in jejunum mucosa for SF and SW ($P \leq 0.10$) compared to d21. At d35 when all pigs had been fed a similar diet for 1 week, plasma, jejunum mucosa and liver Zn concentrations decreased in both M groups ($P \leq 0.09$) compared d21. Liver Zn was higher in MW than MF ($P < 0.01$). For S groups at d35, the only detected effect was a reduction ($P \leq 0.01$) in hepatic Zn concentrations in SF pigs compared with d21 and compared to SW. For Cu at d35, all treatments had lower ($P \leq 0.01$) plasma and liver concentrations compared to d21 whereas both S groups had higher concentrations in jejunum mucosa ($P < 0.03$). In conclusion, pre-weaning Zn and Cu supplementation enhanced Zn and Cu status of pigs at weaning. The reduction in plasma and liver Zn concentrations at d28 and 35 suggests that 300 ppm Zn may not be adequate to maintain Zn status under post-weaning conditions. Independently of the pre-weaning strategy, post-weaning supplementation via drinking water was more efficient than in-feed supply to minimize (not prevent) the transient hypozincemia but it was detrimental to Cu homeostasis. The persistent decrease in liver Cu concentrations, even supplementing 21-fold the NRC recommendations, may be an indication of physiological adjustment, but it needs to be further investigated.

Characterization of a non-invasive indicator for LPS-induced inflammation in the saliva of pigs fed high or low fiber diets

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Saliva has been the focus of a number of pig studies looking for a non-invasive alternative to blood, where indicators of altered metabolic and pathophysiological status could be identified. In the current study, we used an immune stimulation model with lipopolysaccharide (LPS) to alter the metabolic and physiological state of pigs, being fed high (HF) or low (LF) fiber diets. The aim was to identify indicators of immune stimulation in the saliva of LPS challenged pigs with the following selection criteria: (1) diet-independent, (2) significantly different concentration at all post-LPS challenge timepoints compared to the baseline timepoint, and (3) no concentration pattern comparable to control pigs not stimulated with LPS. Therefore, male German landrace littermate pairs (n=22) were fed diets containing 2.8% (LF) or 6.5% (HF) dietary fiber from 50 days of age (d). At 70 d, pigs were fitted with a jugular catheter and transferred to metabolic crates at 74 d. Before the start of the LPS experiment, six of the 44 piglets were excluded from the study due to diarrhea, lethargy or fever, therefore 38 pigs remained in the study. At 75 d, pigs were injected i.m. with either 30 µg/kg bodyweight LPS dissolved in 0.9% NaCl or an equal volume of NaCl as a control, creating 4 groups (HF-LPS, LF-NaCl; n=9/group) and (HF-NaCl, LF-LPS; n=10/group). The study was conducted across 9 experimental blocks with 2-3 littermate pairs per block, and all 4 groups represented. Saliva and blood samples were then taken at -15, 120, 240, 360 and 720 min relative to the LPS injection. In plasma and saliva, free amino acids (n=20), and amino-metabolites (n=13) were measured using HPLC and clinical chemistry parameters (n=10) were measured using an automatic enzymatic analyzer. In blood, hematological parameters (n=19) were measured using an automated Hematology Analyzer, whilst salivary cortisol was measured via ELISA. No blinding was used at any stage of the study. Data were analyzed in R (Version 4.5) using DIABLO (mixOmics) and two Linear mixed-effects models (LMM, lmer). Model 1 included the fixed effect of Time and evaluated time-dependent changes within each experimental group. Model 2 included the fixed effects of Diet, Time and Treatment and their interactions. Sow/Piglet were nested random effects in both Models. The DIABLO analysis showed that at all timepoints after LPS, HF-LPS and LF-LPS pigs differed from the controls in only one parameter, namely salivary 5-aminovaleric acid (5-AVA), a microbial metabolite of lysine. Subsequent LMM analysis confirmed that 5-AVA was significantly higher at all timepoints post-LPS injection and did not change in the NaCl pigs irrespective of diet. In addition, the concentration of 5-AVA was higher in LPS treated compared to NaCl treated pigs, whilst no difference in 5-AVA was observed between HF-LPS vs. LF-LPS and HF-NaCl and LF-NaCl pigs. Due to its stable and diet-independent association with an inflammatory response, 5-AVA could thus be a potential non-invasive indicator of systemic inflammation in pigs. This work is an outcome of the PIGWEB project funded by the European Union's Horizon 2020 research and innovation program under Grant Agreement No 101004770. Ethical consent (LALLF M-V/TSD/7221.3-1-039/21) for all procedures complied to German Animal Welfare Act guidelines and with Directive 2010/63/EU.

Serum magnesium and protein profiles in domestic pigs versus wild boars – possible links to tail biting behaviour

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Establishing reliable reference values for blood parameters are essential for accurate interpretation, especially in wild species where baseline data are scarce. This becomes specifically relevant for comparison purposes to domestic pigs when one wants to reevaluate what is “normal”. For example, in the wild boar population, the behavioural disorder tail biting does not occur and the hypothesis exists that there might be a connection, amongst others, to metabolic differences that might play a role as causative factor. In the present study serum magnesium, total protein and albumin in 135 German wild boars were measured and compared to 60 conventionally reared cross-bred domestic pigs. In the wild boar cohort, age significantly affected magnesium ($p = 0.0012$) and albumin ($p = 0.0170$) but not total protein. Domestic pigs exhibited a markedly significant lower magnesium content with 0.73 mmol/l compared to juvenile wild boars (1.53 mmol/l, $p < 0.0001$) and subadult wild boars (1.37 mmol/l, $p < 0.0001$). For total protein and albumin, a similar pattern could be observed. Domestic pigs showed with 52.71 g/l for total protein and 26.47 g/l for albumin significant lower total protein values compared to juvenile wild boars (total protein: 73.35 g/l, $p < 0.0001$, albumin: 39.28 g/l, $p < 0.0001$) and subadult wild boars (total protein: 74.94 g/l, $p < 0.0001$, albumin: 42.36 g/l, $p < 0.0001$). The magnesium and total protein levels of domestic pigs fell below clinical thresholds for hypomagnesemia and hypoproteinemia, conditions not observed in the wild boar population under study. These findings support the hypothesis that the current management practices around weaning including standard diets according to common nutrient recommendations used in German conventional pig production may leave weaned piglets with critical mineral and protein deficits while no such problems exist in the wild boar population. Such deficiencies could increase post-weaning stress, gut dysfunction and may have a role in the pathogenesis of damaging behaviours such as tail biting.

Haematopoietic traits in indigenous and modern pig breeds are modulated by housing system and developmental stage

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Pig farming is undergoing a process of diversification in order to meet the requirements of animal welfare, sustainability and consumer attitude. In terms of farming systems, these diversified approaches include an enriched housing environment and management practices such as extended suckling periods. In addition, diversification takes into account different breeds with specific characteristics. The interaction of breed, husbandry system and management could bring specific metabolic and immunological benefits that have not yet been sufficiently researched, particularly in the critical early stages of life. Haematological characteristics serve as key indicators of physiological status and immune resilience, providing fundamental insights for the evaluation of diversification. A 2×2 factorial study to assess haematological parameters was conducted using 512 piglets from two breeds (German Landrace, GL; German Saddleback, GS) which were raised under conventional (CON) or organic (ORG) housing conditions. Blood samples were collected at four stages of development, including suckling, pre-weaning, post-weaning and juvenile (70d). Complete blood counts were measured using an automated veterinary haematology analyser. This work was co-funded by the European Union's Horizon Europe project 101136346 (EUPAHW). Marked breed differences were observed with GS pigs consistently exhibiting higher total red blood cell (RBC) counts compared to GL ($P < 0.001$), across all developmental stages. Conversely, GL pigs showed higher counts of mean corpuscular volume (MCV) and mean corpuscular haemoglobin (MCH) at all observed stages ($P < 0.001$). With respect to the leukocyte profile, GS pigs maintained higher total white blood cell (WBC), lymphocyte, and monocyte counts than GL pigs ($P < 0.05$). Post-weaning, GS-CON pigs showed a significant decline in RBC, haemoglobin, and haematocrit (HCT) (4.12%, 6.77%, and 5.24%) compared to GL-CON (1.26%, 3.32%, and 1.92%) respectively, while levels of ORG-housed animals remained more stable. Housing effects at day 70 showed that ORG-housed pigs had reduced HCT and MCV, but significantly higher concentration of MCH compared to CON pigs ($P < 0.05$). Additionally, GL piglets under ORG conditions displayed significantly higher platelet counts (PLT) than GS counterparts ($P < 0.001$). Findings of the haematological profiles point to breed-specific strategies for physiological adaptation in which GS pigs have higher RBC counts but smaller cell size (MCV) putatively to maximise erythrocyte surface area for efficient oxygen uptake, whereas GL pigs possess larger, haemoglobin-rich cells (MCV, MCH). Elevated counts of WBC, LYMPH, and MONO in GS piglets across the developmental period potentially indicate a genetic predisposition for heightened immune surveillance to pathogens. Housing-related differences on day 70, specifically higher MCH and lower MCV under ORG compared to CON conditions, likely reflect compensatory mechanisms for dietary variations, such as methionine availability, thereby affecting erythrocyte maturation. Overall, breed and stage-dependent haematological responses observed underscore functional diversity in physiological and immune adaptation, supporting the concept of matching animal capabilities and needs with housing conditions to enhance resilience and welfare. Ongoing work will utilise transcriptomics to clarify the molecular mechanisms underlying these adaptations and their implications for resilience.

Improving meat quality in local Krškopolje pig breed using advanced optimum contribution selection approach

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Krškopolje pig, Slovenian local breed, is characterised by a relatively high and variable intramuscular fat (IMF) content, a key property determining pork sensory quality. Unfavourable RYR1 C1843T mutation, known to negatively affect water holding capacity, is also present at relatively high incidence. Current breeding programme primarily focuses on the preservation of typical traits related to morphology, coat colour and prevention of inbreeding, while breeding for meat quality, particularly IMF, or reduction of unfavourable RYR1 C1843T frequency, is not yet considered. Therefore, the main aim of this study was to assess the potential for genetic selection for IMF, considering the constraints related to inbreeding and reduction of RYR1 mutation frequency using an advanced optimum contribution selection (aOCS) approach. Genotypic (60K Choice Genetic's Custom SNP-array) and phenotypic data (IMF content) were collected from 455 pigs including the information on farm, age at slaughter and pedigree data. Heritability and breeding values were estimated with 'blupf90+' software using a single-step genomic BLUP approach. Animal model included a random additive genetic effect of the animal, farm (fixed effect) and age at slaughter (covariate). NeEstimator v2.1 was used to estimate the effective population size, and optiSel R package was used to estimate segment-based inbreeding and to apply aOCS. Three scenarios were defined to evaluate different breeding strategies based on selection candidates (animals born from 2021 to 2024): 1) maximising genetic gain for IMF while constraining inbreeding, 2) prioritising the reduction of inbreeding, 3) reducing the RYR1 allele frequency while maintaining inbreeding at the same level. The heritability estimation for IMF content was 0.44 ± 0.10. The segment-based inbreeding coefficient was 0.154 and the effective population size was 46.5, suggesting that Krškopolje pig breed is at risk. The frequency of the unfavourable RYR1 allele was 0.18. As expected, the three scenarios tested revealed different results. The first one highlighted a genetic improvement of IMF (mean of breeding values increased from 0.071 to 0.229) while inbreeding remained stable. However, the RYR1 C1843T allele frequency slightly increased (0.18 to 0.19). The second one showed a decrease in offspring inbreeding (0.200 to 0.181), but also a reduction in expected breeding value (0.071 to 0.033), while the RYR1 allele C1843T frequency remained unchanged. In the last scenario, allele frequency decreased (from 0.18 to 0.15) as did the breeding values for IMF (0.071 to 0.063), while inbreeding remained stable. This study demonstrates that genetic improvement of meat quality is feasible in the Krškopolje pig; however, improvement can be achieved only for one objective at a time. At present, reducing the frequency of the unfavourable RYR1 mutation should be the primary objective of the breeding programme due to its strong effect on meat quality traits, particularly those related to water-holding capacity, while simultaneously ensuring the maintenance of the breed's genetic diversity. Although IMF content is highly variable in Krškopolje pig, its overall level in the population is still relatively high; therefore, we propose that it should be considered as a secondary objective to be addressed after reduction of the RYR1 mutation frequency. Acknowledgements: Funding by Slovenian Research and Innovation Agency (P4-0133, Z4-60178, J4-3094) and project GEroNIMO (EU H2020 GA no. 101000236) are acknowledged.

Computed tomography demonstrates high repeatability when compared to chemical analysis when measuring pork carcass composition

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Objective online technologies are used in commercial abattoirs to estimate carcass composition in pork carcasses. However, the accuracy of these technologies depends on the reference method used in their calibration, which can vary according to its precision and repeatability. To date, no study has been conducted to directly compare the repeatability of Computed Tomography (CT) versus chemical analysis as calibration reference methods for pork carcass composition. Therefore, this study aimed to assess the repeatability of carcass composition measurements taken by CT versus chemical analysis. We hypothesised that CT measurements would demonstrate less variability between repeat scans compared to chemical analysis run on repeat sub-samples. Twenty pig carcasses were selected based on maximising the range of carcass weight (60–80kg) and back fat measurement (P2; 7–16 mm). Carcasses were split into belly, shoulder, loin, and leg sections, CT scanned three times consecutively, and the proportions of lean, fat, and bone % were quantified. Following scanning, the carcass sections were ground, and three subsamples were taken from each section to estimate protein, fat, and ash content. Repeatability was assessed within each carcass section for both the repeated CT measures and the repeated chemical samples using mean absolute deviation (MAD). Supporting our initial hypothesis, CT measurements demonstrated less variability between repeat scans compared to chemical analysis run on repeat sub-samples. For most carcass sections, the MAD for CT measurements of lean, fat, and bone % varied between 0.01 and 0.06 units. The high repeatability of CT measurements observed may be attributed to the robustness of the CT scanning as a non-destructive technique and the use of an additional phantom calibration, beyond the standard calibration. It ensures that tissue measurements remain consistent, independent of external factors such as operator variability and environmental conditions. In contrast, the MAD values for chemical determination of protein, fat, and ash% varied between 0.24 and 0.65 units. This high variability observed in the chemical analysis method likely reflects the influence of destructive sampling procedures, where operator effects during knife dissection of tissues and non-carcass tissues can introduce variations. Additionally, incomplete homogenisation, cross-contamination between fractions, or inconsistencies in solvent extraction and drying procedures may also contribute to the variation. These results indicate that CT is a more repeatable reference method than the traditional chemical estimation of carcass composition.

A standardized workflow for reproducible pig microbiome profiling across laboratories

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The lack of a standardized protocol for DNA extraction in pigs, an animal of biomedical and agricultural relevance, limits reproducibility and cross-study comparability in microbiome research. Technical variance introduced during DNA extraction can obscure biological signals and hinder data integration across studies. Building on a pilot study that identified commercial kits yielding consistent microbial profiles, this study aimed to validate their reproducibility at a larger scale in a multi-center replication study involving six independent research facilities in Germany. We evaluated the effects of three DNA extraction kits on microbial communities using 16S rRNA gene amplicon sequencing and shotgun metagenomics on identical samples processed independently at each institution. Fecal samples from 200 male German Landrace × Pietrain pigs were subjected to DNA extraction using the three kits, following manufacturer protocols at all six facilities. Amplicons targeting the V1-V2 and V3-V4 regions of the 16S rRNA gene and PCR-free metagenomic libraries were sequenced on an Illumina NovaSeq (250 bp and 150 bp paired-end, respectively). Quality control and bioinformatic analyses were performed using DADA2 and QIIME2 for amplicons and Trim Galore with moshpit distributions v2026.1 for metagenomes. Shannon entropy and Bray-Curtis distances were calculated as α- and β-diversity metrics. Two of the three kits maintained stable Shannon entropy across all six facilities in the metagenomic dataset. For 16S amplicons, entropy values were highly consistent across locations (V1-V2: 4.61–5.27; V3-V4: 4.63–5.49), with narrow interquartile ranges and similar medians. Several Kruskal-Wallis tests for location were statistically significant ($p<0.01$), but numerical differences were small and not accompanied by clear shifts in diversity, indicating sensitivity to minor variation rather than relevant biological effects. ADONIS analyses based on Bray-Curtis distances confirmed significant effects of location, extraction kit and their interaction across all sequencing methods ($p=0.001$). However, effect sizes were minimal, location explained 1.5–2.8% of the variance, extraction kit 6.7–8.5% and their interaction 1.3–1.8%, while residual variation accounted for 87–90%. Overall, despite minor statistically detectable compositional differences, microbial community structure was highly consistent across facilities and kits. These results show that the selected DNA extraction kits, combined with the standardized protocol used here, effectively reduce technical variance in multi-center pig microbiome studies. We propose this workflow as a standardized operating procedure (SOP) for future porcine microbiome research, providing a reproducible framework that supports robust cross-study comparability and large-scale meta-analyses.

Improving weaning piglets performance and blood immune parameters by supplementing iron through a milk cup system during lactation

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Iron is an essential mineral for the health and development of piglets, especially during weaning. At birth, piglets have limited iron reserves that deplete quickly as they shift from sow's milk to solid feed at weaning. Iron deficiency can lead to serious health issues, such as iron-deficiency anemia, which negatively affects the growth and overall well-being of the animals (Perri et al., 2016). In the farrowing house, piglets usually recieve iron injected 2d post birth to provide additional iron. Whereas an alternative approach is by providing an iron supplement via the milk replacer. The aim of the trial was to evaluate performance and blood immune cell composition during lactation and its continuity in post-weaning phase in piglets receiving iron injection and additional supplement via the milk replacer. From a farm with 2500 sows equipped with an automated milk cup system (Pump'n'Grow, AB Neo) in farrowing pens, 4 multiparous sows with similar parity were selected. During lactation all piglets (Danbred x Duroc) had access to a creep diet (1.80 ppm Fe) and a fresh milk replacer (Danmilk Supreme®, 150 mg/kg Fe) to supplement sow milk via Pump'n'Grow cup system for 18 days with or without supplementation of 0.2% Piglet Boozt (liquid source of inorganic Fe, 26g/kg). Two days after birth all piglets were injected with Forceris® (1.5 ml containing 133.4 mg/ml of iron III and 355.2 mg/ml gleptoferron). Piglets were weaned at 24 days of age with an average body weight of 6.80 (+/- 1.43) kg. Upon arrival at the nursery facilities (AB Neo Centre of Excellence, Huesca, Spain) all piglets had access to a standard prestarter diet (2.16 ppm Fe) and were weighed at weaning and 15 days post weaning to investigate post weaning performance. Blood samples were taken 9 days pre- and 12 days post weaning for blood immune cell profile during lactation and crucial post weaning period. Pre-weaning treatments were considered as fixed factors in the model. Although weaning weight of the selected litters was significantly lower for the supplemented piglets (6.62 vs 7.73kg) ($p<0.001$), their post-weaning ADG was numerically higher compared to unsupplemented control animals (129.76 vs 110.79 g/d, respectively) ($p=0.134$). Hematocrit increased similarly in both groups between 9d pre and 15d post weaning (4.36 vs 3.15%) ($p=0.213$). Hemoglobin, the protein carrying oxygen in red blood cells tended to be further increased in supplemented pigs (1.07 vs 0.5%) ($p=0.092$), whereas the increase in number of red blood cells (1.25 vs 1.40%) ($p=0.257$) and eosinophils (involved in defense against parasites and allergic reactions) (0.46 vs 0.43%) ($p=0.796$) was similar for both treatments. The increase in monocytes (responsible for phagocytosis of microorganisms and damaged cells) was significantly higher (1.35 vs 0.11%) ($p=0.017$), indicating a better defense in chronic infections and weaning induced inflammation of supplemented piglets. Concentration of basophils and lymphocytes decreased around weaning. Supplemented piglets had a significantly lower decrease for basophils (-2.58 vs –3.14%) ($p=0.011$) indicating better release of histamine in inflammatory response around weaning, whereas no differences were found in the total reduction of lymphocytes (-7.54 vs –7.51%) ($p=0.851$) to fight viral infections and to create immune memory. None of the supplemented piglets required medication in the post-weaning period, whereas 5 of the control animals were treated. In conclusion, supplementing piglets during lactations with a liquid iron supplement (Piglet Boozt – AB Neo) via the milk replacer, resulted in better growth and blood immune status post weaning and lower medication use.

It's not just about feeding. Fatty acid composition of the local Angler Saddleback pig

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Feeding is known to be the main factor influencing the fatty acid composition in adipose tissues of pigs, and in particular the proportion of polyunsaturated fatty acids (PUFA). In a first study using Angler Saddleback (AS) pigs, an endangered breed from Northern Germany, evidence was found that other factors than feeding could be involved. Therefore, a follow-up study was conducted to further evaluate the factors influencing the fatty acid composition in intramuscular fat (IMF) and backfat (BF) and to assess feeding strategies that best meet the lower nutritional requirements and the reduced growth potential of the breed. The study included 58 purebred AS pigs and consisted of two consecutive trials. The first trial involved two feeding variants with different types of roughage (hay vs. grass clover silage). In addition, pigs were fed concentrate reduced in energy (M=12.4MJ) and protein (M=14.2%) as compared to commercial feeding practices. In the second trial, pigs of two further feeding variants both received grass clover silage and two different concentrates, varying in their protein levels (12.3-14.2%), while having the same reduced amount of energy. In addition, the concentrate was fed restrictively from 90kg (first trial) and from 50kg (second trial). Backfat and meat samples were taken from the loin muscle (M. Longissimus thoracis et lumborum) between the 13th and 14th ribs for the analysis of IMF and fatty acid methyl esters (FAME). Using gas chromatography coupled with flame ionization detection, FAME in feed, IMF and BF was analyzed. The BF thickness was determined based on a three-point measurement. With the restriction of concentrate (first trial: M=2.6kg fresh matter/day/pig, SD=0.3; second trial: M=2.5kg, SD=0.1) and protein content, the roughage intake increased across the feeding variants (first trial: M=0.8kg fresh matter/day/pig, SD=0.6; second trial: M=1.6kg, SD=0.9). The BF thickness reached 3.8cm on average in the first trial (SD=0.4), and declined to a mean of 3.5cm in the second trial (SD=0.5) despite the higher age of the pigs (first trial: M=235 days, second trial: M=287 days). The IMF changed only marginally (first trial: M=2.2%, SD=1.3; second trial: M=2.5%, SD=1,0). This was the desired consequence of performance-based feeding with the aim of avoiding excessive fat build-up even during prolonged fattening periods. As expected, the increased roughage intake was accompanied by elevated amounts of PUFA in the diet (first trial: M=45.3%, SD=2.3; second trial: M=58.1%, SD=0.4). However, this was negatively correlated with the PUFA content in IMF (first trial: M=13.8%, SD=3.5; second trial: M=12.4%, SD=2.9) and BF (first trial: M=11.9%, SD=1.3; second trial: M=9.4%, SD=2.1) which was consistently at a low level. This underlines the influence of other factors than feeding. As pigs age, the proportion of adipose tissue generally increases, which is known to be associated with a decline in PUFA levels. However, since the fattening strategies only had a negligible effect on IMF content and actually reduced BF thickness, the influence of age appears to be less relevant here. As in our previous study with AS pigs, a genetic effect with regard to the PUFA level in adipose tissues seems to be reasonable. This may include a special ability to utilize roughage, which has been fed for decades to AS pigs. With this, our study highlights the potential of local pig breeds to make good use of local feed resources and thus provide special meat qualities.

The influence of the sex of fattening pigs on the content of vitamins A and E and cholesterol in the longest dorsal muscle

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Pork remains the most commonly consumed meat in Poland, highlighting the importance of research into its chemical composition and health-promoting properties. Meat is an important source of vitamins, minerals, protein and fat. Vitamins A and E, which are among the most important substances with antioxidant properties accumulated in animal tissues, are of particular significance. The vitamin content of meat depends on many factors, including genetic and environmental influences. Sex can also affect the muscle composition of fattening pigs. The aim of the study was to determine the effect of sex on the content of vitamins E and A and cholesterol in the longest dorsal muscle (musculus longissimus dorsi, LD) of DanBrad hybrid pigs. A total of 30 samples were collected, 10 each from the carcasses of gilts, barrows and non-castrated boars. The content of fat-soluble vitamins A and E and cholesterol was determined in the samples tested. The data collected were subjected to statistical analysis. A one-factor analysis of variance was performed, and the significance of differences between means was determined using Duncan's test and verified at two significance levels: $p \leq 0.05$ and $p \leq 0.01$. The results were statistically compiled using the Statistica PL 13.5 program. The α-tocopherol content (mg/kg) in the longissimus dorsi muscle was 2.026 in gilts, 1.510 in castrated boars, and 1.646 in boars; the β-tocopherol content (mg/kg) was 0.027, 0.022, and 0.016, respectively; the γ-tocopherol content (mg/kg) was 0.136, 0.096, and 0.093, respectively; the Δ-tocopherol content (mg/kg) 0.084, 0.053, and 0.064, respectively; total tocopherols (mg/kg) respectively 2.273, 1.681, and 1.816; retinol content (mg/kg) respectively 0.226, 0.195, and 0.248; and cholesterol content (mg/kg) respectively 28.46, 31.19, and 42.97. When analyzing the vitamin content in the longest dorsal muscle across the individual experimental groups, statistically significant differences were observed in α-tocopherol levels between gilts and surgically castrated boars, as well as in β-tocopherol levels between gilts and boars. Muscle tissue from castrated animals and boars contained significantly less γ-tocopherol than tissue from gilts. No statistically significant differences in Δ-tocopherol content were found in the analyzed muscle between the animal groups. The highest tocopherol (vitamin E) content was found in muscle from gilts' carcasses. Statistically significant differences in vitamin E levels were noted between gilts and castrated boars. Meat from boar carcasses had the highest retinol content, with a significant difference in this component observed between boars and castrated males. Samples of the longest dorsal muscle from boars contained significantly more cholesterol than samples from gilts and castrated males. The differences between groups regarding cholesterol were statistically significant. The results indicate that the sex of the fattening pigs had a significant effect on the vitamins A and E, and cholesterol content in the longest dorsal muscle. Financed by the Minister of Science under the "Regional Excellence Initiative" program

Sensory properties and mechanical parameters of the longest dorsal muscle in fattening pigs depending on sex

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The aim of this study was analyse the influence of the sex of fattening pigs on the sensory properties and mechanical parameters of the longissimus dorsi muscle. A total of 30 samples were collected, 10 each from the carcasses of gilts, barrows, and non-castrated boars. Sensory evaluation of the meat after heat treatment was carried out by a trained panel of five people. In the collected samples, the force and energy required for cutting were determined. The double compression test (TPA) was used to assess hardness, springiness, cohesiveness, chewiness, and resilience of the muscle tissues. The collected data were subjected to statistical analysis, and the results were compiled using the Statistica PL 13.5 program. A total of 30 samples were collected, 10 each from the carcasses of gilts, barrows, and non-castrated boars. Sensory evaluation of the meat after heat treatment was carried out by a trained panel of five people. In the collected samples, the force and energy required for cutting were determined. The double compression test (TPA) was used to assess hardness, springiness, cohesiveness, chewiness, and resilience of the muscle tissues. The collected data were subjected to statistical analysis, and the results were compiled using the Statistica PL 13.5 programme. Analysis of the sensory properties of the longitudinal dorsal muscle showed a significant influence of gender on most of the characteristics studied, with particular emphasis on the desirability of smell and taste, as well as juiciness. The lowest desirability of smell (3.60 points) and taste (3.35 points) was recorded in the group of boars, as confirmed by statistically significant differences ($p \leq 0.05$) compared to gilts and pigs. For taste desirability, these differences were highly significant ($p \leq 0.01$) compared to pigs (4.70 points). At the same time, boars were characterised by the highest taste intensity (4.60 points), which, given the low desirability, suggests the presence of unusual aromas. The juiciness parameter was most favourable in boars (3.85 points) and gilts (3.70 points). Pork meat was significantly less juicy (3.15 points; $p \leq 0.05$) than the other groups. Although no statistically significant differences were found, there was a noticeable tendency towards a higher assessment of the tenderness of gilt meat (3.30 points) compared to boars (2.65 points). Analysis of the physical parameters of the LD-muscle showed a clear distinction between boar meat and the other groups. Boar meat was characterised by the highest cutting force (82.48 N) and cutting energy (217.45 J). These values were statistically significantly higher ($p \leq 0.01$) compared to pigs and gilts. The muscle tissue of gilts was distinguished by the highest elasticity (0.772), cohesiveness (0.712), and springiness (0.326) compared to the other groups ($p \leq 0.05$). The greatest weight losses during heat treatment were recorded in boars (38.34%), which differed significantly from the results obtained for pigs and gilts. When analysing hardness and chewiness, no statistically differences were observed. The highest values for these features were recorded in the longissimus dorsi of boars, and the lowest in pigs. In terms of technology, gilt and pig meat have much better mechanical parameters (lower cutting force) and sensory qualities (higher desirability) than boar meat. The high instrumental hardness of meat from boar carcasses (cutting force and energy) is a significant barrier to their acceptance as a raw material of high culinary quality.
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Evaluation of handheld near infrared spectrometers (NIRS) for prediction of fat quality traits at slaughter

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The fatty acid composition of pig meat and fat is considered a relevant quality trait affecting its nutritional value, sensory and also the further processing properties. Hence, on-site predictions at slaughter would facilitate quality control, decision making and product sorting. Therefore, this project aims to evaluate the feasibility of two handheld NIRS devices that appear to be promising solutions for this goal. Further goal is to find the most appropriate measurement condition (carcass status, body part) for the prediction of fatty acid composition. In a pilot study (PS) 22 carcasses (11 fatty and 11 normal-weight pigs) were chosen for the data acquisition in a slaughter house. Five repeated scans were taken along an approximately 10 cm of the backfat and neckfat (left carcass) using two handheld devices (Trinamix PAL-One – for PS – and PAL-Two later on, and Viavi Micronir OnSite-W). Spectra were obtained both, on hot (about 45 min. p.m.) and cold carcasses (about 24 hrs. p.m.). In the later course of the project, spectra were collected on the neckfat of 430 carcasses (cold, 24 hrs. p.m.), with the pigs originating from two different husbandry systems (conventional, and ecological farming) from Germany. Fatty acid composition of subcutaneous fat was determined in duplicate using GC-FID after fat extraction and preparation of the fatty acid methyl esters (FAME). FAME data (average of the two repeats) were matched with average of NIRS spectra per sample in the case of PS. Multivariate statistical methods were applied after pre-treatment of the spectra (e.g., Savitzky-Golay smoothing and detrending or baseline shift correction). Principal component analysis (PCA) was used for pattern recognition, and partial least square regression analysis (PLSR) was applied for quantitative prediction models of individual fatty acids as well as main groups of fatty acids (saturated -SFA, mono-unsaturated-MUFA, poly-unsaturated-PUFA) using leave-one-out cross-validation. PLSR models were built separately for the back- and neck fat on the two datasets from warm and cold carcasses. Spectral data of the 430 pigs was analysed also with PCA-LDA for the classification of the origin of pigs (husbandry systems). The pilot study revealed effects of location of data acquisition and environmental conditions: backfat width (over M. longissimus) being usually lower than neckfat thickness can result in insufficient spectral acquisition. The window size of the instrument – in some cases – can be bigger than the cut surface (backfat) where spectra are taken. PCA model of PS showed the separation tendency of the spectra of warm and cold carcasses. PLSR models provided better prediction accuracies (R^2CV) in the case of MUFA and PUFA compared to SFA content with both devices. R^2CV of the prediction models ranged from 0.50-0.85, and error value (RMSECV) lower than 2.5% in all cases. PCA models based on cold carcass spectra of 430 pigs did not show a clear separation tendency according to the husbandry system or the slaughter date. However, PCA-LDA classification yielded a clear distinction of the husbandry conditions. Separation trend and better prediction parameters (R^2CV, RMSECV) on the cold carcass could be explained by the cooling process during the warm carcass measurements, which shows the demand for more stable environmental conditions. Regression models provided promising results for the prediction of groups of fatty acids (e.g., PUFA, MUFA), especially with the VIAVI device (950-1650 nm). Further analyses will focus on the extension of the reference dataset and improvement of the robustness. The GlasSchwein project is funded by the German Federal Office for Agriculture and Food (Project ID 28DI107B23).

GlasSchwein: Development of predictive models for parameters of meat quality and quantity along the pork value chain

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The German pork production industry faces increasing economic pressure to improve sustainability and differentiate its products from international competitors. However, existing approaches to digitalize and transfer information between stakeholders that could help to alleviate these problems are often silo solutions, fragmented and lack consistent single-animal traceability. To address these challenges, “GlasSchwein” (“Glass Pig”) is a joint project between partners from industry and academia in Lower Saxony, Germany, that aims to digitalize the pork value chain via a shared data platform connecting relevant actors. By enhancing transparency and active exchange of information throughout the value chain, interoperability is increased, allowing processes to be more easily controlled and optimized. Furthermore, this transparency enables a differentiation of pork products and a targeted improvement of meat quality, facilitating better monetization of individual animals. Piglets are equipped with UHF RFID ear tags, enabling the automatic detection and tracking of each individual pig throughout its entire lifecycle. By utilizing 3D cameras, we obtain automatic daily weight measurements for every pig during the fattening period. In the slaughterhouse, carcass and meat quality traits are assessed using multiple different non-invasive methods, such as ultrasound, near-infrared spectroscopy and hyperspectral imaging. These approaches aim at providing novel quality parameters, allowing farmers to be remunerated for more than just the slaughter weight and lean meat content of their pigs. Additionally, they present new targets for breeding programs to enhance meat quality and increase consumer appeal. A central objective of this project is to utilize comprehensive data obtained along the value chain with its wide variety of data sources to develop predictive models for meat quality and quantity, as well as other relevant metrics, such as the optimal slaughter date. In addition to predicting future parameters, the statistical relationships between them are analyzed. This may provide the opportunity to replace expensive and time-consuming measurements with more cost-effective approaches. Of particular interest is also the impact of pre-slaughter handling and transport between the farm and the slaughterhouse on meat quality, which could create further incentives for improving animal welfare. Preliminary results suggest an association between growth rate of the pigs during the fattening period and different quality measures. However, this association is dependent on the sex of the animals. For example, only sows exhibit a significant correlation between growth rate and lean meat percentage. Notably, this tendency – albeit to a lesser extent – can already be observed after less than a month of fattening. This early indicator provides farmers with the opportunity to potentially influence the meat characteristics by adjusting the type or quantity of feed for individual pigs. However, further analyses are needed to confirm this, along with additional use cases relating to meat quality. The GlasSchwein project is funded by the German Federal Office for Agriculture and Food (Project ID 28DI107B23).

Effect of turmeric supplementation on growth performance, nutrient digestibility, and blood parameters in growing pigs subjected to heat stress

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Heat stress is a major environmental challenge in pig production, negatively affecting feed intake, growth performance, nutrient utilization, and physiological homeostasis. Prolonged exposure to high ambient temperatures induces oxidative stress, inflammation, and immune suppression, ultimately impairing productivity and animal welfare. Natural feed additives with antioxidant and anti-inflammatory properties have gained increasing attention as sustainable strategies to mitigate heat stress–related losses. Turmeric (Curcuma longa), rich in bioactive compounds such as curcumin, has been reported to possess antioxidant, anti-inflammatory, and immunomodulatory effects. Therefore, the objective of this study was to evaluate the effects of dietary turmeric supplementation on growth performance, nutrient digestibility, and blood parameters in growing pigs subjected to heat stress, with the aim of assessing its potential as a nutritional strategy to alleviate heat stress and improve production efficiency. A total of 210 ([Yorkshire × Landrace] × Duroc; 24.3 ± 3.51 kg) growing pigs were assigned to one of three dietary treatments: a basal diet (CON), TRT1, CON + 0.05% turmeric; and TRT2, CON+0.10% turmeric. Data was analyzed in a complete randomized block design using the GLM procedures of SAS (SAS Institute, Cary, NC, USA). Linear and Quadratic polynomial contrasts were used to examine the graded levels (0.05- 0.10%) of turmeric supplementation. . Pigs fed graded level of turmeric supplementation showed linearly improved $P < 0.05$ body weight and average daily gain (ADG) at the end of week 6. Furthermore, pigs fed 0.10 % of turmeric showed tended to improve feed intake with no notable changes on feed conversion ratio (FCR). In addition, nitrogen digestibility was improved in pigs fed graded level of turmeric supplementation. However, there were no notable changes observed on fecal gas emissions or fecal scores. Whereas, blood biochemical analysis revealed significant reductions in serum aspartate aminotransferase (AST) and blood urea nitrogen (BUN) levels, suggesting a potential alleviation of oxidative stress. Overall, dietary turmeric supplementation improved growth performance and nitrogen utilization in finishing pigs and may serve as a nutritional strategy to mitigate heat stress. Keywords: turmeric supplementation; growing pigs, blood profile; oxidative stress

Effect of increasing level of L-carnitine for lactating sows on sow body mobilization, milk composition, litter growth and sow plasma cytokines

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It was hypothesized that increasing levels of dietary L-carnitine for lactating sows would improve the energy utilization of the sow and increase milk production and quality, litter growth and improve body condition and inflammatory status of the sow. In a dose response trial with 6 groups including 245 sows, the sows were fed increasing levels of L-carnitine (0, 179, 294, 532, 734 and 941 mg/kg feed) from 5 days prior to expected farrowing and until weaning at day 28. On day 1 after farrowing the litter was standardized to 15 piglets. The BW and backfat thickness of the sow and the litter weight were measured at standardization and at weaning. In a subsample of 67 sows blood samples from the jugular vein were collected at standardization and weaning and analyzed for IL-6, IL-10 and TNF-α. Milk samples were taken at d 17 of lactation analyzed for DM, fat, protein and lactose and a Brix value was measured. Spline models and linear models were fitted to data and compared for each of the response variables. For all response variables the individual concentration of dietary L-carnitine for each sow was included as fixed effect. All models accounted for a random week batch effect. The final statistical models for analyzing data on changes in sow BW and backfat thickness, litter size and litter ADG included the parameters: sow parity, total litter weight at standardization, sow body weight at standardization, and the sow's average daily feed intake. Models for analyzing milk and blood data included sow parity. P-values below 0.05 were considered statistically significant. The number of weaned piglets (13.6±1.2, P=0.295) and the ADG of the litter (3.1 kg/day, P=0.534) was unaffected by dietary treatment. The loss of back fat was not affected by dietary L-carnitine (P=0.937). For the BW loss the spline fitted better to data than the linear model (P=0.037) and the BW loss was reduced by 3.7 kg with addition of 436 mg L-carnitine pr. kg feed compared to the control group. Milk composition was unaffected by dietary treatment (P>0.05). Plasma IL-6 concentration at litter standardization was best fitted using a spline compared to the linear model (P=0.0303). There was effect of dietary treatment (P=0.0175), but not parity (P>0.05). The plasma IL-6 concentration was highest (2742 pg/mL) at 1002 mg L-carnitine pr kg. Plasma IL-6 concentration at weaning was best fitted using af spline compared to the linear model (P=0.0219). There was effect of dietary treatment (P=0.0132), but not parity (P>0.05). The plasma IL-6 concentration was highest (2735 pg/mL) when fed 1002 mg L-carnitine pr kg. IL-10 concentration at standardization was best fitted using a spline model compared to the linear model. There was effect of dietary treatment (P=0.0341) but not parity (P>0.05). The IL-10 concentration was highest (76.4 pg/mL) at 748 mg L-carnitine pr kg. The plasma TNF-α concentration at standardization and weaning and IL-10 at weaning was not affected by dietary treatment (P>0.05). A reduction in the BW loss could have a positive impact on the following reproduction, but it is not likely that a difference of 3.7 kg would affect reproduction negatively. I Higher dietary L-carnitine increases metabolic rate, fat mobilization, and milk production, all of which naturally elevate metabolic IL-6 signaling after farrowing and during lactation. The rise in IL-10 reflects a normal anti-inflammatory compensatory response balancing increased IL-6. In conclusion, only minor effects were seen on sow plasma cytokines concentrations and sow BW loss when feeding increasing levels of L-carnitine during lactation.

Effect of increasing level of extruded linseeds for lactating sows on changes sow body mobilization, litter gain, milk composition and plasma cytokines.

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It was hypothesized that increasing levels of dietary omega-3 fatty acids for lactating sows can improve milk production and quality, litter growth and improve body condition and inflammatory status of the sow. In a dose response trial with 6 groups in total including 208 sows, the sows were fed increasing levels of extruded linseed (0, 1.2, 2.4, 3.6, 4.8 and 6.0 % of Easylin) from 5 days prior to expected farrowing and until weaning at d28. On d1 after farrowing the litter was standardized to 15 piglets. The BW and backfat thickness of the sow and the litter weight were measured at standardization and at weaning. In a subsample of 56 sows blood samples from the jugular vein were collected at d6 and 17 and analyzed for IL-6, IL-10 and TNF-α. Milk samples were taken at d 6 and 17 of lactation and analyzed for DM, fat, protein and lactose using MilcoScan, as well as fatty acid composition and a Brix value was measured. Spline models and linear models were fitted to data and compared for each of the response variables. For all response variables the individual daily intake of α-linolenic acid (ALA) for each sow was included as fixed effect. All models accounted for a random week batch effect. The final statistical models for analyzing data on changes in sow BW and backfat thickness, litter size and litter ADG included the parameters: sow parity, total litter weight at standardization, sow body weight at standardization, and the sow's average daily feed intake. Models for analyzing milk and blood data included sow parity. P-values below 0.05 were considered statistically significant. The BW loss (P=0.573) and mobilization of back fat (P=0.473) was unaffected by inclusion of extruded linseed. Number of weaned piglets was not affected by dietary treatment (P=0.620), but there was a tendency for increased litter ADG with 22 g/d per 1% unit extruded linseed (P=0.090). Milk fat % at d6 was best fitted using a linear model and linear model was different from the null model (P=0.0409). There was effect of dietary treatment (P=0.0333). An increased intake of 1 g ALA increased the fat % by 0.029. The ALA content (4.71 mg/100 g milk) was optimized at 33.47 g of dietary ALA per day. Milk protein % at d17 was best fitted using a spline model. There was effect of dietary treatment (P=0.0088). The milk protein % was optimized (4.57%) at a daily intake of ALA at 21.5 g/d. The n-6:n:3 ratio of the milk at d17 was best fitted using a spline model. There was effect of dietary treatment (P<0.001). The n-6:n-3 ratio of the milk was decreased with increasing dietary ALA and was lowest at 41.5 g of dietary ALA per day with a n-6:n-3 ratio at 2.50 and highest at 8.0 g of dietary α-linolenic acid per day with a n-6:n-3 ratio at 10.9.. The small increase in litter ADG could be a result of increased content of fat and n-3 fatty acids in the milk. Plasma IL-6 at d17 was best fitted using a spline model compared to the linear model and there was effect of dietary treatment (P=0.0087) . The lowest daily intake of ALA (8.23 g/day) resulted in the highest plasma concentration of IL-6 (16358 pg/mL). Plasma IL-6 at d6, IL-10 and TNF-α was not affected by dietary treatment (P>0.05). High dietary ALA lowers plasma IL-6 because it reduces arachidonic-acid–derived inflammatory eicosanoids and activates anti-inflammatory transcription factors. In conclusion, increasing dietary levels of ALA altered the milk composition and the inflammatory status of the sow, but only a tendency for increased litter ADG.

Lactation feed intake and feed intake relative to body weight are associated with litter weaning weight across sow parities

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Lactation is an energetically expensive process for sows, requiring sufficient feed intake (FI) to support milk production, litter growth and maintenance of sow body condition (or growth for gilts) for subsequent parities. Lactation FI and water intake (WI) are known to be associated with litter performance. However, both vary between sows due to body size and across parities. Thus, making absolute intake difficult to interpret across sows. Expressing intake, relative to sow body weight may provide a size-adjusted indicator to determine if lactation intake is adequate. This study aimed to quantify lactation FI and WI influence on litter weaning weight from consecutive parities. We hypothesized that the novel metric, lactation FI relative to sow body weight (FI:BW ratio) would be positively correlated with litter weaning weight across parities 1-5, and WI would increase with increasing lactation feed intake. This study was conducted at AFBI pig research farm. 43 sows (landrace x large white) were monitored over 5 consecutive parities (1-5). Sows and their piglets were housed in conventional individual farrowing crates. From the day of farrowing, food and water were provided to sows Daily FI (kg), WI (L), were recorded for each individual sow. On the day of weaning (28 days post-farrowing) sows and their respective litters were weighed. Statistical analyses were conducted using R (Version 4.3.1). Linear mixed-effects models included sow as a random effect to account for repeated measurements across parities. Fixed effects included either total lactation FI or FI:BW ratio, parity, and total piglets weaned. Additional models evaluated associations between WI, FI, and litter weaning weight. Significance was set at $P<0.05$. Interactions between intake variables and parity were tested and were not significant. Total lactation FI ranged from 112 to 240 kg and total lactation WI ranged from 348 to 1052 L. Litter weaning weight decreased with increasing parity relative to parity 1 ($P < 0.01$). Approximately 14% of the variation in litter weaning weight was attributable to between-sow differences. Total lactation FI was positively associated with total litter weaning weight ($\beta = 0.196 \pm 0.039$ kg per kg feed; marginal $R^2 = 0.577$; conditional $R^2 = 0.635$; $P < 0.001$). Each additional kilogram of feed consumed during lactation increased litter weaning weight by approximately 0.20 kg. Similarly, there was a significant positive relationship between FI:BW and litter weaning weight (marginal $R^2 = 0.568$; conditional $R^2 = 0.627$; $P < 0.001$), demonstrating that higher FI relative to sow size supported increased litter weaning weight. Water intake was associated with FI ($\beta = 2.16 \pm 0.40$; marginal $R^2 = 0.262$; conditional $R^2 = 0.640$; $P < 0.001$), indicating that sows consuming more feed also consumed more water (mean 3L:1kg feed ±0.59). However, WI alone was not significantly associated with litter weaning weight ($P > 0.05$), suggesting that its influence on litter performance may be driven by FI. These findings confirm the importance of maximising sow FI during lactation to support litter growth. While WI increased with FI, it did not independently predict litter weaning weight in the current dataset. These results support the hypothesis that FI:BW ratio was positively associated with litter weaning weight across parities and may be a useful metric to assess adequacy of size-adjusted intake in lactating sows.

E. coli and early mortality: Association of MUC4, MUC13 and FUT1 parental genotypes with pre-weaning mortality in a commercial pig population

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Diarrhoea caused by enterotoxigenic Escherichia coli (ETEC), particularly F4 and F18 strains, remains an important cause of mortality in pigs and results in substantial economic losses. Genetic resistance to ETEC infections has been associated with SNP markers located near candidate genes such as MUC4, MUC13 and FUT1, which are involved in intestinal mucin biology and host receptor glycosylation. While these markers have been proposed as indicators of host resistance through the absence of functional receptors for specific fimbriae (F4 and F18), their relevance for pre-weaning mortality (PWM) under commercial production conditions is not yet fully established. The objective of this study was therefore to evaluate associations between parental genotypes at three E. coli–related loci (MUC4, MUC13 and FUT1) and PWM. The dataset consisted of 739 genotyped hybrid sows and 1370 genotyped Piétrain boars used on a commercial farm in Flanders, Belgium. In total, 1381 litters with complete parental genotypes and phenotypic records for piglets born alive and pre-weaning mortality were available. Genotype distributions were assessed for Hardy–Weinberg equilibrium. Exploratory analyses included genotype frequencies and calculation of mean PWM across genotypes and parental genotype combinations. To account for the binomial nature of the data, generalized linear mixed models with a logit link were fitted, modelling the number of pre-weaning deaths relative to the number born alive. Models included parental genotype (or genotype combination) as fixed effect, parity and gestation length as covariates and sow and boar as random effects to account for repeated observations. For MUC4 and FUT1, no significant associations were detected at sow level, boar level, or parental genotype combination level ($p > 0.05$). In contrast, for MUC13, no significant effects were observed when analysing sows or boars separately, but parental genotype combinations were significantly associated with PWM ($p = 0.0309$). For the MUC13-related marker ASGA0091537, the C-allele has previously been associated with resistance to F4 fimbriae adhesion. In the present study, litters from CT×CT (odds-ratio = 0.75, $p = 0.0012$) and CT×TT (odds-ratio = 0.82, $p = 0.0125$) parental genotype combinations (boar x sow) showed significantly lower mortality compared with the reference combination TT×TT. Random effects indicated substantial variability between sows (σ^2sow = 0.136) and boars (σ^2boar = 0.072), suggesting that individual animal effects remain important determinants of PWM. Overall, these results indicate that under commercial conditions, MUC4 and FUT1 genotypes are not strongly associated with pre-weaning mortality. However, parental genotype combinations at the MUC13 locus may influence early piglet survival, potentially reflecting non-additive or heterozygote-related effects. It should be noted that the present study only included records on pre-weaning mortality, while some of these markers have primarily been associated with resistance to post-weaning diarrhoea. Although the observed effects are moderate, these findings highlight the importance of evaluating marker effects under practical production environments and considering parental genotype interactions when assessing candidate genes for ETEC resistance.

Rethinking AI for Animal Welfare: Framing, Misinformation and Ethics

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Animal welfare is a complex, multi-faceted concept, and how ”good welfare” is defined often depends on the values and priorities of various decision-makers. Artificial Intelligence (AI) is increasingly framed as a tool for monitoring and improving farm animal welfare, but is this framing justified? Recently, we systematically examined how the welfare impacts of precision livestock farming (PLF) are framed in the literature and found that most studies used positive language, implying that technology use improves animal welfare. However, despite this positive framing, few studies were able to draw a clear connection between a specific PLF measure and a concrete welfare outcome. Instead, much of the language remained vague, falling short of establishing a link between a given technology and a welfare improvement, indicating “welfare-washing”. Equally limited was the discourse surrounding the ethical deployment of technology in livestock farming and the potential welfare risks that PLF use may introduce. This “welfare-washing” bias extends beyond the scientific literature. Using ChatGPT’s text-to-image model, we show how general purpose AI can reinforce misinformation about livestock farming to the broader public through hidden internal mechanisms. The model consistently romanticized farming, depicting dairy cows grazing on pasture and pigs rooting in mud, even when explicitly prompted for realistic depictions of industrialized farming. The root cause of this bias lies in the model’s internal prompt revision process, hidden from users, which rewrites inputs to produce more aesthetically appealing images. When we bypassed this process, the base model demonstrated clear awareness that most farm animals in industrialized countries are housed intensively, producing images that accurately reflected modern farming practices (i.e., pigs housed on concrete). Our work shows that hidden internal mechanisms within AI have the potential to erase the reality of controversial topics like intensive livestock farming. These types of algorithms can further exacerbate the disconnects between public perception and actual farming practices, hindering meaningful dialogue addressing disconnects between farming with societal values, and undermining the social sustainability of both AI and livestock farming. These concerns are further compounded by the hidden costs of AI itself. Many PLF solutions are now being scaled up using AI, but AI is not a value-neutral tool. Criticisms include its massive energy consumption during training and inference time, mining of rare minerals and natural resources to build the physical infrastructure hosting these digital systems. Concerns also exist regarding the exploitation of labour from marginalized communities for data labeling and review of toxic content, and large-scale scraping of internet data without consent or regard for copyright. From a One Welfare perspective, the environmental and societal harms associated with on-farm AI use may not be offset by animal welfare improvements, even under conditions where such improvements are well-established. Moving forward, we propose adapting the 3Rs framework, the internationally accepted ethical principles guiding the humane use of animals in science, for use when integrating AI into livestock farming. We suggest researchers consider: 1) replacement: should AI be used at all? 2) reduction: minimize the number and size of models, computations, and iterations while maintaining scientific integrity and, 3) refinement: include validation steps and improve energy efficiency in both training and deployment.

The Digital Ethogram: A Multimodal, High Time-Resolution Representation of Animal Behaviour

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Ethograms have long served as the foundational framework for describing animal behaviour, primarily based on human visual observation and historically focused on species-specific motor patterns. Human observational capacity often limits ethograms to behaviours with an onset lasting 1 to 10 seconds. Advances in video recording have improved the quality of behavioural observations by better controlling intra- and inter-observer reliability and reducing the possible impact of the observer on the animal's behaviour. The use of wearable sensors and acoustic monitoring has expanded the ways we can capture animal behavioural data. However, these technologies are often applied as extensions of existing observational paradigms rather than as catalysts for redefining behavioural representation itself. The advent of these high-frequency, multimodal data streams raises the question of whether traditional ethogram structures are appropriate for fully appreciating the subtlety and complexity of behavioural data, especially when destined for analysis by Artificial Intelligence (AI). A simple behavioural transition from lying to standing is traditionally coded as a single event with a lone timestamp. However, high-frequency digital monitoring devices enable the description of this transition by hundreds of data points or more. Such a transition from lying to standing can be decomposed into distinct phases, revealing behavioural structure that often is not visible within traditional ethogram frameworks. Furthermore, the synthesis of digital data streams from multiple types of time-resolved detectors further extends the observational landscape and offers even richer datasets. Here, we introduce the concept of a digital ethogram and describe the metrics required for synchronized video, inertial measurement units, and acoustic data to enable the representation of behaviour across multiple temporal and biological scales. Digital ethograms retain the interpretability of classical ethology while enabling higher resolution measures of latency, duration, intensity, asymmetry, and behavioural sequencing. By pairing annotated behavioural labels with multimodal sensor signatures, these representations yield reproducible datasets well-suited to machine-learning applications while remaining grounded in biological meaning. Importantly, this approach shifts the focus from isolated behavioural snapshots to continuous behavioural streams, enabling the detection of health- or welfare-relevant patterns, such as early indicators of discomfort or subtle changes in movement quality, with greater robustness. By reframing ethograms as compilations of structured temporal representations rather than solitary human-coded observations, digital ethograms derived from multimodal, high-temporal-resolution data promise to support more nuanced, scalable, and biologically meaningful insights into animal behaviour, welfare, and health. Digital ethograms do not replace human observation, but extend it, providing a scalable framework for behavioural science in the era of big data, precision livestock farming, and AI-enabled health and welfare assessment and insights.

Health and Welfare Commission perspectives on AI and farm animal welfare

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The rapid integration of artificial intelligence (AI) into livestock systems presents both transformative opportunities and significant challenges for animal welfare. As precision livestock farming evolves, AI-driven tools—such as real-time monitoring, predictive analytics, and automated decision support—offer potential to enhance early disease detection, optimize management practices, and improve individual animal outcomes in increasingly intensive production environments. These uses remain largely focused on increasing production efficiency and performance rather than on improving animal welfare. The Health and Welfare (HW) Commissions view is that technologies must not serve merely as efficiency enhancers that perpetuate confinement-based systems or mask underlying welfare deficits. We believe that welfare science must play a critical role as AI becomes more deeply embedded, ensuring that technological advancements prioritise ethical considerations, holistic well-being, and the "One Welfare" framework over reductionist fixes. Key concerns include the risk of further alienating animals from stockpeople, reducing sentient beings to data points, and reinforcing large-scale intensive models through economic dependencies on high-tech interventions. By centring welfare expertise at the intersection of ethics, science, and emerging technologies, the HW Commission advocates for responsible AI deployment that aligns with consumer expectations, scientific evidence on the benefits to welfare of facilitating natural behaviours and pasture access, and broader societal demands for humane livestock production. This perspective provides an essential anchor for balanced discourse, guiding the livestock sector toward innovations that genuinely advance animal welfare rather than merely sustaining current paradigms.

From data streams to breeding values: developing and harmonizing novel phenotypes for sustainable livestock genetic improvement

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The rapid expansion of data collected in livestock production systems, including genomic information, on-farm records, sensor-derived measurements, and other high-throughput phenotypes, offers unprecedented opportunities to refine animal breeding programs. However, the effective use of these data requires careful consideration of how traits are defined, measured, standardized, and integrated across diverse data sources. This presentation will discuss key steps involved in the development and implementation of novel phenotypes for genetic improvement. First, emphasis will be placed on the identification and definition of biologically meaningful traits derived from emerging data streams. Such traits must be evaluated for data quality, repeatability, and genetic parameters to determine their suitability for inclusion in breeding programs. Second, the importance of harmonizing data structures and standardizing phenotypes across farms, recording systems, and technologies will be highlighted, as inconsistencies in measurement protocols and data formats remain major barriers to large-scale analyses. Examples from dairy cattle and other livestock systems will illustrate how integrating phenotypes from traditional recording systems with information derived from sensors and other technologies can support the study of complex traits related to health, welfare, efficiency, and resilience. Particular attention will be given to approaches and tools for integrating heterogeneous datasets, ensuring data quality, and investigating the genetic architecture of newly developed traits. Finally, the presentation will discuss how well-defined and standardized phenotypes can be incorporated into genetic evaluations and breeding objectives, ultimately enabling the development of more informative selection tools. These topics are highly relevant to researchers, breeding organizations, and industry stakeholders seeking to translate rapidly expanding data resources into practical genetic improvement strategies.

Data standardisation for welfare and genetics evaluations

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Today, a wide range of sensors and monitoring systems are rapidly expanding to measure additional animal characteristics, expanding the opportunities to collect data related to the welfare of animals, their individual and group behaviour, robustness and sustainability performance. These technologies generate a new volume of data which can be used to effectively prevent harmful events, to support early detection tools, breeding strategies and targeted interventions in individual animals, farms or at the population level. To be able to widely implement such strategies and tools, those systems and the data collected need to seamlessly communicate to deliver reliable, robust, high-quality, and comparable data. ICAR plays a central role in building scientific and practical consensus for establishing joint international guidelines for harmonization, validation and certification of devices, methods and exchange of data. Achieving consensus on trait definitions, reference standards, and data usage is essential to ensure consistency and value. This multidisciplinary collaboration brings together expertise from across the dairy value chain, helping translate cutting-edge academic research into practical tools and real-world applications. As sensor technologies continue to evolve, ICAR is creating the framework needed to evaluate new devices, to facilitate interoperability and data flows, and harmonize how their results are interpreted. Several initiatives and projects have emerged in this context.

European Network on Livestock Phenomics: an international initiative aimed at promoting and resourcing advanced phenotyping in livestock

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Phenomics aims to systematically characterize the phenome, namely the set of observable physical and molecular traits of an animal. In livestock systems, high-resolution phenotypic data generated through precision livestock farming (PLF) technologies, imaging, sensors, and omics approaches are increasingly central to breeding, management, health, and welfare decisions. However, the rapid expansion of data-intensive phenotyping raises major challenges related to data management, interoperability, standardization, ethical use, and the integration of artificial intelligence (AI) tools. The European Network on Livestock Phenomics (EU-LI-PHE; COST Action CA22112) was established to foster multidisciplinary collaboration addressing these challenges. This contribution presents the structure, objectives, and ongoing activities of EU-LI-PHE, with particular emphasis on ethical, regulatory, and data-governance aspects of advanced livestock phenotyping within the evolving AI landscape. EU-LI-PHE brings together approximately 500 members from more than 50 countries, spanning animal breeding, PLF, genomics, bioinformatics, engineering, economics, and social sciences. The Action is structured into five Working Groups (WG): WG1 (Phenotyping technologies) addresses existing technologies and infrastructures and defines roadmaps for high-dimensional, whole-animal data acquisition. WG2 (Genome-to-phenome integration) consolidates knowledge on genomic and epigenomic contributions to phenotypic variation and identifies priorities for translational applications. WG3 (Computational resources and data analysis methodologies) advances computational models, analytical tools, computational infrastructure, and AI frameworks for large-scale data management and interpretation. WG4 (Economic impact, regulation, policy, and societal aspects) evaluates economic implications, regulatory and data-governance frameworks, and societal acceptance. WG5 (Stakeholder engagement, communication, and dissemination) ensures coordinated stakeholder interaction and dissemination across all WGs. Within this framework, particular attention is devoted to enhance data interoperability across species and to achieve FAIR data principles. A great importance is also given to ethical and legal implications of large-scale data and to the societal acceptance of AI-driven models in decisions. Capacity building is supported through training schools, Short-Term Scientific Missions, Virtual Mobility Grants, and targeted conference grants, with strong emphasis on inclusiveness and early-career development. Since 2024, numerous mobility and dissemination grants have strengthened collaboration and skills in big data analytics and responsible innovation. By integrating technological, computational, ethical, and policy perspectives, EU-LI-PHE contributes to defining a coordinated European roadmap for livestock phenomics. The network aims to ensure that AI-enabled phenotyping in PLF systems develops within a robust framework of data standardization, responsible governance, and societal trust, ultimately supporting sustainable and resilient livestock production. Acknowledgment: EU-LI-PHE (CA22112) is funded by the European Union – COST European Cooperation in Science & Technology.

SENSTARA: Advancing sensor-based indicators of animal resilience, health, and welfare in precision livestock farming

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Livestock systems are increasingly challenged by climate change, production intensification, and growing societal expectations regarding animal welfare, environmental sustainability, and transparency. In this context, Precision Livestock Farming (PLF) technologies offer powerful tools for continuous animal monitoring. However, translating large and heterogeneous sensor datasets into robust, biologically meaningful indicators that can be consistently applied across species, production systems, and environmental conditions remains a major challenge. SENSTARA is an international Working Group within the EAAP Precision Livestock Farming Commission that aims to advance sensor-based assessment of animal resilience, health, and welfare in livestock systems. Through advanced sensing technologies, modelling, and artificial intelligence, SENSTARA promotes the development, validation, and harmonization of sensor-derived indicators, metrics, and models, thereby improving methodological standardization across species and production systems. Its scientific and technological scope encompasses wearable, environmental, and physiological sensors; imaging technologies such as infrared thermography and video-based monitoring; acoustic and environmental sensing systems; and advanced data analytics, modelling, and artificial intelligence approaches. By fostering collaboration among researchers, technology developers, industry stakeholders, and policy actors, SENSTARA provides an open and interdisciplinary platform to accelerate the translation of PLF innovations into practical, transparent, standardized, and scalable decision-support tools.

Data preprocessing transparency as a requirement for robust sensor based livestock indicators

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Precision Livestock Farming (PLF) technologies generate continuous behavioural and physiological data that hold substantial potential for advancing the assessment of animal performance, health, and welfare. In addition to animal-borne devices, many research and commercial settings also rely on production and environmental monitoring systems, gas-measurement technologies (e.g., methane measuring units), imaging platforms, acoustic sensors, and location-tracking systems. However, a major but often overlooked challenge is the lack of transparency in how commercial systems handle raw sensor data before delivering research-ready outputs. Typically, the protocols describing procedures for data cleaning, filtering, aggregation, interpolation, and anomaly handling are undisclosed. These undocumented preprocessing steps directly affect the reliability, comparability, and biological interpretability of sensor-derived indicators. In practice, customers, including farmers, advisors, and researchers, may receive only aggregated daily values, without access to underlying high-resolution data or knowledge of how missing values, outliers, or device failures were addressed. Such limitations create risks for biased assessments, restrict the integration of multi-sensor information, limit harmonisation across sensors, species, and production systems, and undermine reproducibility, especially when validation studies rely on special high-resolution datasets not accessible in routine use. A growing number of sensor technology manufacturers openly provide raw data at the highest resolution available, enabling transparent and standardised preprocessing within research workflows. Yet wide variation persists across sensor types, species applications, and commercial platforms. The EAAP PLF Working Group SENSTARA (Sensors and Standards Development for Research Activities) sheds light on these discrepancies, aiming to raise awareness of their scientific implications and promote minimum expectations for data transparency. We propose a collaborative effort, including fact sheets, standard operating procedures for data cleaning, and community dialogue, to develop pragmatic, species-agnostic guidance that supports more robust and interpretable use of sensor-derived indicators across the full spectrum of PLF technologies. This contribution invites researchers, developers, and industry partners to engage with SENSTARA in shaping a more transparent and reproducible future for PLF research.

Effect of milk replacer formulations on goat kids' growth performance and small intestinal development.

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The pre-weaning milk feeding is an essential nutritional phase and one of the main investments for the rearing of goat kids. Different substitutes, rather than goat milk (i.e. whole cow milk, cow milk replacer, milk whey), have been used in dairy goat farms, with cow's milk (whole or replacer) being the most profitable option (Zamuner et al., 2023). However, the differences in composition of goat milk compared with cow milk (+30% fat, +19% protein, +16% total solids,-8% lactose) could affect the growth and health performance of the kids consuming cow's milk, with special concerns around the lactose levels that could potentially lead to gastrointestinal effects (diarrhea or bloating). This experiment aimed to investigate the effect on growth performance and small intestinal development of goat kids fed different goat and cow milk powder formulations. 416 male goat kids were randomly assigned at birth to 1 of 4 treatments: Goat milk powder (GP; n = 100), Cow Milk powder (CP; n = 105), Goat mix (GM; n = 103), or Cow Mix (CM; n = 108). The GM was formulated to mimic the CP components, while the CM reached the GP components. The GM included lactose (8.5%) and whey protein (3%); the CM included cream (22%), whey protein (27%) and lactose (4%). Goat kids had ad libitum access to their assigned milk formulation until weaning (5 weeks). Body weight (BW) was recorded at birth, and weeks 2, 5, 7, and 9 weeks old. At week 5, a subset of 40 kids (n = 10/group) were slaughtered. Hot and cold carcass weights were recorded, and duodenum samples were collected for histology analysis. The villi length (VL), villi weight (VW), crypt depth (CD), muscular thickness (MT), epithelium thickness (ET) and number of villi per cm (NV) of the duodenum samples were measured using Image J software (version 1.54 Wayne Rasband and National Institutes of Health, USA). All data were analysed using mixed model with repeated measure in SAS (Statistical Analysis System; version 9.4.01). From 2 weeks old onward, the GP and CM formulations had better growth performance (144 g/d vs 100g/d; P<005). At 7 weeks, kids fed GP (13.0±0.1kg) and CM (12.5±0.1kg) were 18% heavier than those fed CP (11.0±0.1kg) and GM (11.4±0.1kg; P<0.05). The GP kids had 11% greater hot and cold carcass weights compared to kids fed CP and GM (P<0.05); no differences were shown between GP and CM carcass weights (P>0.05).There was a tendency for the villi length in the GP group to be longer than in the other groups (P=0.07), while no differences were found between groups for VW, CD, MT, ET and NV. Overall, kids fed the CM formulation have a similar growth performance than those fed the GP formulation, with the addition of being a cost-effective alternative for dairy goat farms. The similar growth performance of the GM with the CP formulation suggests that the level of lactose included in these formulations could influence goat kid performance. The histological results suggested no differences in the duodenum development under the four powder formulations. Further analysis into circulating metabolites (including glucose, total protein, and non-esterified fatty acids) will provide a better understanding of the metabolic implications of the milk powder formulation of goat's overall performance. It was concluded that CM and GP formulations are effective feeding alternatives that commercial producers can implement.

Impact of Colostrum and Serum Derived IgG with Cream or Vegetable Oil on Growth and Immune Status of Dairy Goat Kids

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Colostrum plays a crucial role in providing passive immunity to newborn dairy goat kids by supplying maternal IgG and IgM antibodies shortly after birth. While most studies on colostrum focus on antibody concentration and timing of feeding, less attention has been given to the colostrum matrix, particularly the fat phase in which antibodies are embedded, and how this matrix affects IgG absorption, metabolism, growth, and immune function. The objective of this study was to determine how different combinations of IgG source (colostrum vs. serum) and fat source (colostrum cream vs. vegetable oil) influence the efficiency of IgG absorption, passive immunity, and growth performance. Forty-four newborn dairy goat kids were randomly assigned at birth to one of four colostrum or colostrum-replacement treatments: pooled whole colostrum (PC; n = 12; body weight; BW 2.98 ± 0.12 kg), pooled skimmed colostrum supplemented with vegetable oil (PO; n = 10; BW 3.13 ± 0.15 kg), adult goat serum formula with colostrum fat (SF; n = 11; BW 2.75 ± 0.20 kg), or adult goat serum formula with vegetable oil (SO; n = 11; BW 2.61 ± 0.15 kg). Each kid received its assigned treatment at 10% of BW during the first 24 h of life, divided into several meals. Blood samples were collected at 24 and 48 h of age to determine serum IgG and IgM concentrations, and BW was recorded at birth, 30 and 60 days of age to calculate average daily gain (ADG). Health status and general vigor were monitored throughout the experimental period to detect any clinical signs related to treatment. Data were analyzed using a mixed model (JMP Pro), with colostrum source and age as fixed effects, kid as a random effect, and a repeated-measures structure for variables measured over time. Body weight was affected ($P< 0.01$) by colostrum source, with the highest BW at 60 days observed in kids fed SF (8.43 kg) and the lowest in those fed SO (6.82 kg), whereas PC and PO kids showed intermediate BW (7.84 kg). As expected, BW increased from 30 to 60 d of age, and ADG was greater ($P< 0.03$) during the first 30 days (125 g/ d) than from 30 to 60 days (113 g/ d), indicating a decline in growth rate with age. A partial analysis (n = 5) of serum IgG concentration showed that levels at 48 h (8.14 mg/ mL) were lower than at 24 h (9.23 mg/ mL), reflecting the normal decline following gut closure, and IgG concentrations were numerically lowest in the SO group (5.72 mg/ mL) compared with the other sources (9.67 mg/ mL). These results suggest that, under the conditions of the present study, IgG absorption at 24 h was not primarily determined by the origin or nominal concentration of IgG (serum vs. colostrum), but was influenced by the type of fat present in the colostrum or formula matrix (colostrum cream vs. vegetable oil). The findings highlight the capacity of serum-based colostrum formulas, particularly when combined with colostrum cream, to support substantial passive immunity, growth rate, and BW in dairy goat kids, offering a potential strategy when maternal colostrum is limited or variable. At the same time, replacement of colostrum cream with vegetable oil in serum-based formulas appeared to compromise growth and IgG status, pointing to the importance of matching both the immunological and nutritional components of natural colostrum. Further research is warranted to elucidate the mechanisms by which colostrum fat composition modulates IgG transport across the gut and to optimize colostrum-replacement formulations for commercial dairy goat systems.

Effect of breed on rumen microbiota in lambs treated with aqueous extract of Tithonia diversifolia as an additive

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The use of additives in animal nutrition, such as monensin, has been relevant due to the emergence of antibiotic-resistant microorganisms. The inclusion of plant extracts, such as that of Tithonia diversifolia (TDE), is presented as an alternative to improve ruminal microbiota and animal health. This study analyzed how the breed of lambs influences the response to these treatments in terms of bacterial diversity and abundance. Twenty-eight lambs from the Katahdin, Santa Inês, and Pelibuey breeds, with an average weight of 19.03 ± 4.57 kg, were randomly assigned to four treatment groups: Control (no additives), Monensin (20 mg/day), TDE (5 g/day), and TDE (10 g/day), for a duration of 42 days. Ruminal fluid was collected at the time of slaughter, and an analysis of bacterial composition. For bacterial community analysis, raw read quality was inspected with FastQC and processed in QIIME 2, including import, visualization, quality filtering and removal of chloroplast and mitochondrial sequences using the DADA2 algorithm. From the amplicon sequence variant (ASV) table, a relative abundance table was generated and used to estimate taxon abundances at phylum, family and genus levels based on the SILVA v138 database. Microbial community data were analyzed and visualized in R, importing the abundance table with taxonomic assignments into the phyloseq package and generating plots at genus and family levels with ggplot2. To minimize bias from low-abundance features, ASVs with fewer than 5 reads and samples with fewer than 5,000 reads were removed prior to downstream analyses. Permutational multivariate analysis of variance (PERMANOVA) was used to test for differences in multivariate community composition. The results demonstrated that breed had an impact on the differential composition of the ruminal bacterial community. The breeds exhibited variations in the abundance of certain phyla and bacterial genera. For instance, lambs of the Katahdin breed showed a higher abundance of Ruminococcus in the TDE10 treatment, suggesting an enhancement in fiber degradation, contributing to increased production of propionate and butyrate. In contrast, Pelibuey lambs exhibited a greater presence of Rikenellaceae in the TD5 treatment, which may favor acetate production. Lambs of the same breed exhibited consistent trends in ruminal bacterial community abundance, displaying similar abundance profiles across treatments. This breed-specific microbial patterning aligns with prior research demonstrating host genetic effects on microbiota. In conclusion, based on our results, the effect of breed on ruminal microbiota is evident, with specific differences in the abundance of bacterial groups in response to the treatments. These findings suggest that interactions between diet, type of additive, and breed may influence the efficacy of treatments in the development of microbiota, highlighting the necessity to consider breed genetics when implementing additives in ruminant feeding.

Replacing corn grain with dried sugar beet meal in fattening goats: effects on feed efficiency, gastrointestinal microbiota and plasma metabolomic profile

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In Mediterranean and North African production systems, identifying drought-tolerant, cost-effective feed resources is essential to reduce reliance on irrigated cereal crops such as corn. Sugar beet represents a promising alternative energy source for small ruminants. This study evaluated the effects of replacing corn with dehydrated ground sugar beet roots in fattening Murciano-Granadina goat kids, a breed widely reared in Mediterranean regions. Twenty-four 5-month-old male kids were allocated to three dietary treatments (n = 8 per group) and fed complete pelleted diets ad libitum for 49 days. The experimental diets contained either 18% corn (CTRL), 9% corn and 9% dehydrated ground sugar beet roots (SB09), or 18% dehydrated ground sugar beet roots (SB18). The sugar beet ingredient consisted of dehydrated ground sugar beet roots (90% DM), with 7.6% crude protein and 12.4% NDF (DM basis). Feed intake, growth performance, nutrient digestibility, rumen fermentation parameters, the composition of ruminal, fecal, and salivary microbiota, and circulating metabolic, oxidative, and inflammatory markers were evaluated. Plasma metabolomic profiling was performed to explore systemic metabolic responses to dietary treatments. Sugar beet inclusion reduced feed intake and average daily gain ($p < 0.05$), whereas dry matter digestibility was not affected. Ruminal pH and total volatile fatty acids remained stable across diets, while butyrate and valerate increased in SB-fed animals ($p < 0.05$). Ruminal microbiota showed dose-dependent responses: SB09 increased bacterial richness and evenness, whereas SB18 reduced richness and promoted sugar-fermenting taxa (e.g., Olsenella, Candidatus Endomicrobium) at the expense of fibrolytic bacteria (e.g., Treponema, Roseburia). Fecal and salivary microbiota did not differ among treatments. SB diets were associated with lower circulating insulin levels, whereas oxidative stress indicators (AOPP, TBARS, FRAP) and inflammatory markers (leptin and lactoferrin) were not influenced by diet. Preliminary metabolomic analyses revealed metabolic shifts associated with dietary treatments, highlighting pathways potentially linked to energy and lipid metabolism. Dehydrated ground sugar beet roots can be included in fattening goat diets without impairing ruminal stability or systemic oxidative status. However, high inclusion levels negatively affect intake and growth performance. Moderate inclusion appears to be the most effective compromise, maintaining microbial diversity while limiting performance losses, and may represent a practical alternative to corn in Mediterranean and North African goat production systems. Keywords: Sugar beet; goat kids; feed efficiency; ruminal fermentation; gastrointestinal microbiota; plasma metabolomics

Feeding hydroponic barley sprouts to early lactation dairy goats: consequences for intake, nutrient utilization, milk performance, and immune markers

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Dairy production in arid and semi-arid regions is increasingly constrained by climate change, chronic water scarcity, and limited availability of high-quality forages, thereby stimulating interest in alternative, water-efficient feed resources such as hydroponically produced barley sprouts (BS). The objective of this study was to evaluate the effects of including BS in total mixed rations (TMR) for early-lactation dairy goats on feed intake, nutrient digestibility, milk yield (MY) and composition, as well as selected blood and immune parameters. All the TMRs for the different dietary treatments were prepared at commercial feed center four weeks before the experiment began. After mixing and chopping in a stationary mixing wagon, the diets were tightly packed and sealed into plastic to ensure anaerobic conditions. The TMRs included wheat silage and a heterofermentative inoculum, and all rations including the SB diet were fermented and stabilized before and during the experimental period. Thirty-six multiparous dairy goats, which were consuming wet TMR under standard farm management practices (mean ± SE: 3.01 ± 0.17 kg/d MY and 35 ± 12 DIM at the beginning of the trial), were allocated to one of three TMR groups after a 2-week adaptation phase and observed for 6 weeks. The treatments included a control diet and two experimental diets where BS harvested at either 5 or 7 days replaced parts of the dietary CP, starch, and NDF, while ensuring the overall chemical composition of the diets remained similar. Furthermore, SB mainly replaced the wheat silage, leading to adjustments in roughage NDF by altering the quantities of alfalfa and oat hay in the diets. Milk yield was recorded daily, and milk composition was determined weekly. In the final week of the experiment, feces, rumen fluid, and blood were sampled to assess apparent total-tract digestibility and rumen and blood traits; additional blood samples were collected in week 3 and 6 to quantify peripheral leukocyte expression of immune-related genes. Data were analyzed using mixed models including diet, time, and their interaction as fixed effects, and goat nested within diet as a random effect, with repeated measures over time. Dry matter intake (DMI) was greater ($P< 0.01$) in goats fed the control diet compared with sprout-fed goats (2.60 vs. 1.53 kg/ d), and both MY and energy-corrected milk (ECM) were higher ($P< 0.004$) in the control (MY= 3.05 kg/d) than in the BS diets (MY= 2.31 kg/d). In contrast, milk fat, protein, and lactose concentrations did not differ among treatments, and feed efficiency (ECM/DMI) was not affected by BS inclusion. Apparent digestibility of organic matter and gross energy was lower ($P< 0.01$) in control goats than in goats fed BS, indicating improved energy utilization with sprout inclusion despite reduced intake. Expression of HSP70 in peripheral blood leukocytes was greater ($P< 0.05$) in week 3 in goats fed the 5-d BS diet, whereas IL1b expression did not differ among diets but decreased from week 3 to week 6 ($P< 0.05$), suggesting a time-related modulation of inflammatory signaling. In conclusion, high inclusion of hydroponic BS in TMR for early-lactation dairy goats reduced feed intake and milk production despite higher organic matter and energy digestibility, and was associated with altered HSP70 expression. These findings suggest that bioactive compounds present in hydroponic sprouts may influence intake regulation and immune-related responses, highlighting both the potential and the limitations of BS as an alternative feed resource for dairy goats in arid regions.

Milk and plasma multi-omics: insights into feed conversion and residual intake in dairy goats.

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Improving feed efficiency (FE) in dairy production systems is important for reducing input costs and improving sustainability. Feed conversion ratio (FCR; the ratio between feed intake and milk production) and residual feed intake (RFI; difference between actual and predicted feed intake for a given production level) are common estimates of FE. Additionally, measuring FE on a large scale is costly and time consuming, requiring individual feed intake and production data. Thus, high throughput tools to screen animals for FE are attractive. Metabolomics and lipidomics provide powerful tools for understanding the physiological mechanisms underpinning FE traits. This experiment aimed to investigate the differences in omics' profiles between early lactation dairy goats divergent for FE (FCR vs. RFI). From 54 early-lactation Saanen does (12 mo old), dry matter intake (DMI; daily), body weight (BW; three times weekly), milk yield (daily) and milk composition (twice weekly) was recorded for 49 d. The DMI and energy-corrected milk (ECM) were used to calculate FCR (DMI/ ECM). The RFI was calculated as regression residuals of DMI on metabolic bodyweight and ECM. Metabolomic and lipidomic profiling was carried out on 32 does (selected on divergence for RFI), with focused comparisons of the seven most extreme high- (H-RFI; high efficiency, low RFI values) and low- (L-RFI; low efficiency, high RFI values) RFI animals (n = 7/group) and a separate n = 7/group comparison based on FCR (H-FCR and L-FCR) to investigate pathway differences. Phenotypic data was subject to REML analysis using GenStat (22nd ed). The omics data was normalised by median, log10-transformed, and analysed based on peak intensities using MetaboAnalyst 6.0 (https://new.metaboanalyst.ca). Pathways with $P < 0.05$ and false discovery rate (FDR) < 0.20 were considered significant. Area under curve (AUC) > 0.8 and $P < 0.05$ were considered candidate biomarkers for individual lipids and metabolites. Six goats overlapped between efficiency classifications: 4 were H-RFI and H-FCR, 1 was L-RFI and L-FCR, and 1 H-RFI but L-FCR. The oPLS-DA models explained a greater proportion of the variation of RFI in both milk and plasma compared to FCR (RFI: 14–32%; FCR: 15–23%). The H-RFI and L-RFI goats had no differences in BW and ECM ($P > 0.05$). However, the H-RFI (high efficiency) goats consumed 0.4 kg DM/d less feed than the L-RFI (low efficiency) goats ($P < 0.05$), highlighting the independence of RFI from production traits. Alternatively, the H-FCR goats had a greater ECM than L-FCR (3.7 vs 2.6 kg/d; $P < 0.001$), lower BW (44 vs. 52; $P < 0.05$), but no differences in DMI ($P > 0.05$). This suggests that genetic selection based on FCR could unintentionally alter other physiological characteristics. Metabolomic pathway analysis revealed 6 differential pathways distinguishing divergence in FCR (including galactose metabolism) and 2 for RFI (including biosynthesis of unsaturated fatty acids) in milk and plasma. Glutathione metabolism was of significance in both RFI and FCR ($P < 0.02$; FDR < 0.20). Across milk and plasma, 59 candidate biomarkers were identified for FCR and 75 for RFI. In our experiment it was concluded that distinct metabolic profiles were evident between FCR and RFI in early lactation dairy goats, attributed to differences in amino acid and energy metabolism.

Associations of residual feed intake with growth performance, body size, carcass traits, meat quality and blood parameters in growing Naimi lambs

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Residual feed intake (RFI) is a key efficiency trait that can reduce feeding costs and improve the sustainability of sheep production. This study evaluated how divergence in RFI is associated with growth performance, body size, carcass traits, meat quality and blood parameters in Naimi sheep. We hypothesized that lambs with low RFI would consume less feed without impaired growth and would display shifts in carcass, meat and blood profiles compared with high RFI lambs. Forty male lambs (59.8 ± 4.3 d of age; 18.9 ± 1.3 kg BW) were subjected to a 15-d adaptation and a 63-d test period and were fed diet containing 30% forage (Alfalfa hay) and 70 % concentrate on DM basis with individual recording of feed intake and growth. Residual feed intake was calculated for all lambs during the test, and animals were ranked retrospectively and classified as most-efficient (M-EFF; n = 18, RFI = −0.06 ± 0.03) or least-efficient (L-EFF; n = 22, RFI = 0.05 ± 0.04). Data were analyzed using mixed models with RFI group as fixed effect in SAS 9.4. Initial and final BW, backfat thickness and average daily gain did not differ ($P > 0.05$) between groups, whereas M-EFF lambs had 10% lower ($P = 0.01$) average daily dry matter intake than L-EFF lambs. The M-EFF lambs had greater ($P = 0.02$) rump depth and tended ($P \leq 0.10$) to have greater shoulder width and body proportionality and area indices. However, the M-EFF lambs had lower ($P = 0.01$) loin girth and tended ($P \leq 0.10$) to have shorter ear length, smaller muzzle diameter, and lower pelvic and dactyl thorax indices. Carcass traits indicated that M-EFF lambs had thicker body walls ($P = 0.02$), larger neck base diameter ($P = 0.05$) and wider foreshank ($P < 0.01$), and tended ($P \leq 0.10$) to have greater testis and rack measurements, while reduced ($P = 0.01$) loin and leg bone measurements. Regarding meat quality, M-EFF lambs had greater ($P < 0.01$) muscle pH at 0 h post-mortem and tended ($P = 0.09$) to have greater pH at 24 h with a tendency ($P = 0.07$) for greater meat cohesiveness. Most-efficient lambs also exhibited greater ($P \leq 0.05$) blood pCO_2, glucose, hematocrit and hemoglobin and lower ($P = 0.03$) blood pH, together with altered red blood cell and leukocyte indices. In conclusion, M-EFF lambs maintained similar growth with lower feed intake compared with their L-EFF counterparts. Furthermore, M-EFF group showed greater body wall thickness and lower loin and bone proportions. They also showed alteration in post-mortem muscle pH and in blood variables related to oxygen-carrying capacity and metabolic status. These findings indicate that improved feed efficiency in desert-adapted lambs is associated with shifts in tissue distribution, muscle pH and hematological profile, supporting the use of RFI for sustainable genetic improvement.

How diet can improve lamb muscle lipid profiles and rumen microbiome

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Omega-3 fatty acids are a type of polyunsaturated fatty acid found in various foods, which have been shown to have numerous health benefits when consumed in optimal amounts. These benefits include promoting cardiovascular health, supporting cell structure, and modulating the inflammatory response. Rich sources of n-3 PUFA include oily fish, seeds, nuts, and brassica vegetables. The lipid profiles and fat content of meat products can be influenced by two main factors: genetics and diet. Grass is a rich source of alpha-linolenic acid (C18:3 n-3), a precursor to longer chain fatty acids, but its deposition in muscle is limited due to significant hydrogenation in the rumen. The use of feed supplements to increase n-3 PUFA levels is also limited by the toxicity of certain long-chain PUFA to rumen microbiomes. Microalgae, on the other hand, has been shown to have protective properties that allow LC n-3 PUFA to bypass rumen processes without being damaged. This study investigates how animal diet impact fatty acid composition, specifically omega-3 PUFA, and the concentration and distribution of lipid species across classes. The aim of this study was to investigate the effect of lamb diet on production performance, muscle lipid profiles and effect on rumen microbiome. Lipid extracts from Longissimus and Leg muscle samples (n=26) from grass, concentrate and high microalgae as well as grass and concentrate treatments, respectively, were reconstituted in 900 µL 20% of mobile phase B (mobile phase B was 90% isopropanol, 10 mM ammonium formate and 0.1% HCOOH in ultrapure water) and 100 µL chloroform and centrifuged. 3 µL was injected into the UHPLC/MS system. Lipids were then directly analyzed using a Vanquish UPLC-System (Thermo Scientific, Waltham, MA, USA) with a heated electrospray ionization (HESI) QExactive plus Orbitrap mass spectrometer (Thermo Scientific, Waltham, MA, USA) in positive ion mode. Chromatographic separation took place on a reversed-phase column (Accucore Polar Premium 100×2.1 mm (2.6 µ) with guard column: Accucore Polar Premium 10×2.1 mm (2.6 µ)) from Thermo Scientific (Waltham, MA, USA). Identification and quantification of individual lipid species were performed by LipidSearch Software 5.1 from Thermo Scientific (Waltham, MA, USA) on product level (MS/MS fragmentation). At the start of the study, all the ruminal volatile fatty acids (VFA) were similar between the control and the microalgae treatment. At time of the slaughter propionic acid was the only VFA with significant difference between both treatments and the microalgae treatment lowered the propionic acid concentrations from 24 to 15 mmol/L. A methane reduction of 23% was estimated by microalgae treatment compared to control. The number of bacteria and archaea genera present in gastrointestinal samples during the study decreased similarly across the different treatments. The results of the untargeted LC-MS lipidomics study of lamb muscle diet treatments shows that C18:3 n-3 lipid species are mainly incorporated into phospholipids of Longissimus and Leg muscle after grass diet treatment, but also into TGs of exclusively Leg muscle. The algae diet treatment leads to a considerable increase of C22:5 and C22:6 n-3 in specific phospholipids and TGs that is not seen in the grass diet treatment.

The supply with branched chain volatile fatty acids during the transition period and the lactation of dairy ewes.

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The aim of the present study was to investigate the effects of supplying branched-chain volatile fatty acids during the transition period and in lactation in dairy ewes. Forty pregnant Karagouniko breed ewes were allocated into ten pens of four animals each, based on expected lambing date and anticipated litter size. One month before the expected parturition, one of two treatments were randomly assigned to each pair of similar pens: (1) control, without supplementation (CTR), and (2) dietary supplementation with 4 g/day of a feed additive containing branched-chain volatile fatty acids (IsoFerm, Zinpro; ISF). The study lasted until 60 days postpartum and included three periods: (1) prepartum, (2) suckling period up to 40 days postpartum, and (3) lactation period. Dry matter intake before and after parturition did not differ between treatments, although it differed significantly between time periods (on average 1.45 and 2.25 kg at 21 days before and 21 days after parturition, respectively; $P < 0.001$). Litter size was not different between CTR and ISF ewes and averaged 1.75 lambs/ewe. Lamb birth weight was higher in ISF ewes, and lamb body weight continued to be higher for ISF lambs until weaning, where ISF lambs resulted 2.1 kg heavier (or 14.8 % heavier; $P = 0.04$) compared with CTR lambs. The overall average daily gain was higher in lambs nursed with dams fed ISF (282 vs 245 g/d for ISF vs CTR lambs, respectively; $P = 0.04$). Milk yield at 55 days postpartum tended to be 10.9% higher in ISF ewes, whereas milk protein concentration was lower compared to CTR ewes (5.42 vs. 6.48% for ISF and CTR, respectively; $P < 0.05$). A similar decreasing trend was observed only in the percentage, but not in the total yield, of whey protein β-lactoglobulin (β-LG). Furthermore, specific alterations were observed in milk fatty acid profile, which were mainly associated with changes in the animals' energy metabolism due to ISF supplementation. Previous studies have demonstrated that ISF supplementation improved NDF rumen degradation providing higher metabolizable energy, reducing body reserves mobilization during transition. In conclusion, IsoFerm supplementation improved the energy metabolism of dairy ewes during the periparturient period, leading to increased milk production and lamb body weight and growth until weaning.

Comparing weaning strategies: growth performance of dairy goat kids in intensive systems

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Weaning carries important economic, management and nutritional consequences for dairy producers. Depending on production type, dairy goat farms set different target criteria (e.g. age, weight, solid feed intake) and employ either abrupt or gradual weaning strategies. Early weaning strategies can reduce milk-feeding costs, particularly when milk-based diets and labour are expensive. Despite the effect of age at weaning being explored in goat kids, only a few have investigated weaning methods from artificial milk-feeding systems, and only one focused on the pen level. This experiment aimed to investigate the effects of 5 different weaning methods on growth performance in female goat kids at the pen level. Four week old female goat kids (n=270) were randomly assigned to one of 5 treatments (n = 54/ group): abrupt weaning at 5 weeks (ABW5), or 6 weeks (ABW6), diluted weaning (DIL; concentration of milk replacer was reduced by 20%/ each day with ad libitum feeding), restricted access (RES; access to milk feeder for 4 hrs/ day from week 5 for 7 days), diluted and restricted access [DILRES; combination of reduced milk replacer (DIL) and limited access (RES)]. Daily intake (dry matter intake; DMI) of milk replacer, pellets and straw was recorded daily from weeks 4 to 7, with ad libitum access to solid feed in all the groups. Pellet and straw were introduced to the kids at 2 weeks old, but consumption was recorded only from week 4. The body weight (BW) was recorded at birth, then weekly from week 4 to 8 and again at 12 weeks old. All data were analysed using a mixed model with repeated measures in SAS (Statistical Analysis System; version 9.4.01), with treatment as a fixed effect, kid ID as a random effect, and initial BW as a covariate. Initial body weight at 4 weeks old did not differ between groups. At week 6, the ABW6 (11.8 kg) and DIL (11.4kg) groups were 10% heavier than the kids in ABW5 (10.2 kg), REST (10.5 kg) and DILREST (10.2 kg) groups ($P<0.001$). However, at week 7 (one week after all groups were weaned), no differences were found in BW (SED=±0.15; $P>0.05$). At 8 weeks old, kids in the RES group were lighter than those weaned at ABW6 (12.1 vs 12.7; $P<0.01$), with no difference between the other treatments. At 12 weeks, the DILRES (16.8 kg) group was 3% lighter than those weaned under DIL, ABW6 and RES (17.4 kg; SED=±0.15; $P<0.01$), but similar to those under the ABW5 method. The DMI varied between groups during weeks 5 to 6 according to access to the milk replacement. However, from week 6 to 7, when all the groups were weaned, the DMI was greater in kids weaned in the ABW5 group. The total DMI from week 4 to 7 weeks was numerically lower for DILRES (7.1 kg/kid) than ABW5 (7.9 kg/kid), RES (8 kg/kid), ABW6 (8kg/kid) and DIL (8.4 kg/kid). Two kids (0.7%) died of pneumonia during the experiment. Overall, kids weaned under an abrupt (ABW5 or ABW6) or gradual (DIL, REST and DILREST) weaning method had a similar BW 1-2 weeks post-weaning. However, kids weaned using the DILRES method were 3% lighter 6 weeks post-weaning at 12 weeks old. From a growth-performance perspective, all examined weaning methods are suitable for use at the pen level on intensive dairy goat farms. The choice of weaning method should consider the costs and intake of milk replacer and solid feed. Further investigation into the impact of weaning methods on the circulating metabolites (e.g. glucose, total protein and non-esterified fatty acids) during and after weaning, and on feed intake beyond the first week post-weaning is warranted.

Assessment of feeding rations in small ruminant farms using the Small Ruminant Nutrition System

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Nutrition plays a critical role in productivity, health, and welfare of small ruminants, hence also affecting farm profitability. The objective of this study was to assess nutritional management practices and feeding rations in commercial small ruminant farms in the region of Eastern Macedonia and Thrace, Greece. Data were collected from 37 farms using a standardized questionnaire documenting general farm characteristics, nutritional management strategies, and ration formulation. Feedstuff and bulk tank milk samples (n=3) were collected from each farm for chemical composition analysis. Feeding rations for milked animals were evaluated using the Small Ruminant Nutrition System (SRNS), a mechanistic model that predicts nutrient requirements. Inputs included the physical and chemical composition of rations, the chemical composition of bulk tank milk, and average milk production data per animal. The DMI input was based on the daily feed offered per lactating animal as reported by the farmer. Grazing was not included in the SRNS evaluation, as no farm practiced grazing during the first three months of lactation. Descriptive statistics were computed using R programming language. A formal nutritional plan was absent in 45.9% of farms, while 37.8% relied on feedstuff company protocols. Farms used period-specific feeding rations during the dry period (94.4%), steaming up (86.1%), and flushing (61.1%). Average starch content was 33.7% ± 3.15% and concentrate to forage ratio was 1.5 ± 0.42. On average, dietary energy supply exceeded modeled requirements by 62.3% in sheep and 53.5% in goats, whereas protein oversupply averaged 70.5% and 111.2%, respectively. Mean rumen degradable nitrogen supply corresponded to 123.5% and 121.9% of requirements in sheep and goats, respectively. Estimated urea production energy cost in sheep and goats was 0.177 Mcal/day and 0.190 Mcal/day, respectively. Overall, the findings indicate higher energy and protein supply relative to animal requirements in both sheep and goats, contributing to elevated costs and potential animal health risks. These results emphasize the need for improved farmer training and evidence-based ration formulation. This work is part of a research project funded by the Region of Eastern Macedonia & Thrace (ELKE-AUTH:76290).

Exploring the ruminal metabolome of sheep supplemented with essential oils

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Understanding rumen function is key to improving feed efficiency, reducing methane emissions, minimizing antimicrobial interventions, and ultimately promoting the sustainability of ruminant production. Dietary interventions are central to rumen manipulation, as they represent an accessible and rapid approach to modulate rumen function. However, despite decades of research, a large proportion of the rumen metabolome remains uncharacterized, limiting our understanding of the metabolic responses to dietary strategies. This study aimed to characterize the ruminal metabolome of sheep fed essential oils from garlic or rosemary. Thirty-five crossbred lambs were assigned to five treatments: control, garlic low-dose (30 mg/kg DM; GL), garlic high-dose (216 mg/kg DM; GH), rosemary low-dose (86 mg/kg DM; RL) for 105 days, and rosemary high-dose (216 mg/kg DM; RH). At slaughter, rumen fluid samples were collected from three animals per treatment. The rumen fluid was centrifuged, and the cell-free supernatant was subjected to two extraction methods with either ethyl acetate or methyl tert-butyl ether. Ultra-high-performance liquid chromatography coupled with tandem mass spectrometry, operated in both positive and negative ionization modes, was used to obtain fragmentation data. The resulting data were processed using the Global Natural Products Social (GNPS) platform to generate a molecular network. Compounds were putatively annotated by comparing fragmentation spectra with the GNPS database, public spectral libraries, or through manual annotation. A total of 7,064 chemical entities were detected in the rumen fluid overall, of which 47 compounds were putatively identified through molecular matches in public databases. When ethyl acetate extraction was used, 3,269 chemical entities (46.28% of the total) were detected, of which 12 were putatively identified. The chemical entities exclusive to each treatment were distributed as follows in negative mode: no additive, 64; GL, 29; GH, 92; RL, 81, and RH, 81; and in positive mode: no additive, 111; GL, 72; GH, 458; RL, 145; and RH, 151. When methyl tert-butyl ether extraction was used, 3,795 chemical entities (53.72% of the total) were detected, of which 35 were putatively identified. The chemical entities exclusive to each treatment were distributed as follows in negative mode: no additive, 64; GL, 83; GH, 57; RL, 9; and RH, 43; and in positive mode: no additive, 223; GL, 688; GH, 340; RL, 105; RH, 157. Despite the large number of putative metabolites, only 0.67% of the detected compounds matched existing spectral libraries. This study provides a snapshot of the rumen fluid metabolome of sheep fed different essential oils at different concentrations. Less than 1% of the detected chemical entities matched available spectral libraries, highlighting the vast untapped potential of the rumen for the discovery and characterization of novel compounds. Key words: garlic, essential oils, rosemary, rumen metabolome, sheep, untargeted metabolome

Sex-specific effects of local and imported alfalfa on performance, intake, digestibility, morphology, methane emissions, and nitrogen balance in Korean crossbred goats

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We hypothesized that imported alfalfa would improve nutrient utilization and reduce enteric methane emissions compared with local alfalfa due to potentially lower ash and fiber contents resulting from differences in harvest maturity and agronomic practices, and that these responses would differ between female and male Boer × Korean native crossbred goats (Capra hircus) raised in Korea. Both forages were alfalfa hay (not dehydrated pellets). Eight mature Korean crossbred goats were used, including four females (initial BW 40.9 ± 1.4 kg) and four males (48.6 ± 1.3 kg). Treatments were arranged in a repeated 2 × 2 factorial design with forage source (local vs. imported alfalfa) and sex (female vs. male) as main effects. Diets were formulated at a forage-to-concentrate ratio of approximately 55:45 and adjusted to body weight to meet growth requirements. After a 7-day cage adaptation period, followed by a 30-day dietary adaptation period in individual cages, the goats were transferred in stages to metabolic chambers for measurement of gas exchange and nitrogen balance. Growth performance, nutrient intake, apparent digestibility, morphometric traits, methane (CH_4) and carbon dioxide (CO_2) emissions, and nitrogen utilization were evaluated. Data were analyzed using the PROC MIXED procedure of SAS, with forage source, sex, and their interaction included in the model; significance was declared at $p<0.05$. Chemical composition analysis revealed that imported alfalfa had lower ash (5.7 vs. 11.4%) and neutral detergent fiber (45.5 vs. 51.8 %) than local alfalfa, despite similar crude protein levels. Sex markedly influenced intake, excretion, and gas emissions. Male goats had greater total dry matter intake than females (38.2-38.3 vs. 27.0-31.2 kg), accompanied by higher fecal output and greater CH_4 energy loss (290.6-327.2 vs. 108.1-169.0 kcal/d). Males also produced higher absolute CH_4 and CO_2 emissions, consistent with their greater intake and metabolic activity. Apparent digestibility, particularly of fiber fractions, was improved in goats fed imported alfalfa, with the response being more pronounced in females. Female goats fed imported alfalfa showed the lowest CH_4 (11.6 g/d) and CO_2 (508.1 g/d) emissions, suggesting improved fermentation efficiency and lower enteric energy loss. Male goats fed imported alfalfa also had lower CH_4 (21.9 g/d) and CO_2 (1038.3 g/d) emissions than males fed local alfalfa. In contrast, nitrogen retention and nitrogen use efficiency were higher in females than in males. Morphology also improved under imported alfalfa, particularly in males, with greater body length, withers height, chest width, and chest girth. These results indicate that the observed advantages of the imported alfalfa were attributable to its specific compositional profile rather than origin per se, and that imported alfalfa is not universally superior to local alfalfa. Overall, forage source and sex jointly influenced nutrient utilization and enteric emissions in Korean crossbred goats. Imported alfalfa improved digestibility and reduced gaseous losses, especially in females, indicating that sex-specific feeding strategies based on measured forage quality may enhance production efficiency while mitigating environmental impact in goat systems.

The lactation and seasonal effects on glucose utilization in dairy goats under high ambient temperature

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Background: Lower milk yield has been demonstrated in both dairy cows and goats raised under tropical areas. High ambient temperature (HTa) is the major external factor influenced milk synthesis by mammary gland. Lactose is the main milk component supporting milk yield. Much evidence suggested that glucose utilization is influenced by HTa conditions. Objective: The present experiments have focused on the effect of HTa on glucose utilization using intravenous glucose tolerance test (IVGTT). The objective of this experiment is to investigate whole-body glucose utilization in dairy goats fed under tropical conditions. Methods: An IVGTT was performed in dairy goats at different physiological stages including lactation, mid- and late- pregnancy (n=5 each). Next, we investigated the effect of natural HTa on IVGTT in dairy goats from two different seasons: summer and winter (n=8 each). Results & discussion: Early lactation goats had higher basal blood glucose concentration (43.2 ±1.9 mg%) and lower the area under curve from IVGTT (6584 ±459 mg%.min) than those mid- and late- pregnancy (35.4 ±3.4 and 36.3 ±2.7 mg%; 8170 ±851 and 8659 ±530 mg%.min, respectively). The results suggested that during early lactation basal plasma glucose and glucose uptake capacity might be prioritized to support milk synthesis. This indicates an important role of the homeorhetic control on mammary gland functions. Next, the average noon ambient temperatures (Ta) were 35.7 ± 1.6 ˚C (winter) and 38.7 ± 1.0 ˚C (summer). With the Ta difference at 3 ˚C between winter and summer seasons (seasonal ΔTa), neither the rectal temperature (39.1 ± 0.1 and 39.3 ± 0.1 ˚C, respectively) nor the IVGTT curve (8283 ± 918 and 9197 ± 1012 mg%.min, respectively) differed significantly between seasons. Although the IVGTT protocol from the current experiment could demonstrate the effect of lactation on the whole-body glucose utilization, the test failed to differentiate the seasonal HTa effect with seasonal ΔTa at 3 ˚C. This possibly comes in part from the small difference in Ta between winter and summer. Conclusion: Whole-body glucose utilization, as measured by IVGTT, is significantly different during the transition from pregnancy to lactation. A wider range of seasonal ΔTa is needed to investigate the effects of natural HTa on glucose utilization in dairy ruminants.

Technologies for Individual Sensing of Poultry in Commercial Hen Houses

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Precision livestock farming increasingly relies on sensing technologies capable of monitoring individual animals within commercial production systems. In poultry houses, however, most sensors measure environmental conditions at the house level rather than the physiological or behavioral state of individual birds. Because modern poultry houses may contain tens of thousands of animals, such average measurements may not accurately represent the conditions experienced by individual hens. Individual sensing is therefore essential for early detection of welfare problems, improved climate control, and adaptive flock management. This study presents several complementary technological approaches for non-invasive sensing of individual poultry in commercial housing systems. The first approach focuses on physiological monitoring using infrared thermography. A sensing unit equipped with a low-cost infrared camera was installed near feeders to estimate the body temperature of individual broilers during voluntary visits. Thermal images of the head region were automatically processed and body temperature was predicted using a regression model based on image-derived features. Validation against implanted temperature loggers showed that the system can estimate body temperature with an accuracy of about ±0.27 °C. Continuous measurements indicated that the thermal state of individual birds may differ from environmental temperatures measured by conventional house sensors, highlighting the potential of individual sensing to improve heat-stress detection and management. The second approach addresses behavioral monitoring in cage-free laying hen houses using artificial intelligence based computer vision techniques. High-resolution cameras installed in a commercial house were used to collect large-scale video data of flock activity. Deep-learning models detect and track individual hens and identify behavioral events characterized by rapid coordinated movements that are associated with aggressive chasing behavior. The detection system achieved about 90% accuracy in identifying individual birds and enables automated alerts indicating possible harmful interactions within the flock. A third example explores the use of artificial intelligence for optimizing cooling perches designed to mitigate heat stress in laying hens. Computer vision algorithms analyze the positions and postures of hens resting on perches to detect resting or lying behavior and quantify perch utilization patterns. Combined with environmental measurements, these observations allow estimation of hens' preferences for different perch cooling conditions and support adaptive management of perch-based cooling strategies. Developing individual sensing systems for poultry presents challenges that differ from those encountered in monitoring large farm animals. Poultry houses contain very large populations with high stocking densities, resulting in frequent occlusions and making continuous identification of individuals particularly challenging. The small body size of hens and the economic constraints of poultry production also limit the feasibility of wearable sensors. These constraints suggest that future poultry monitoring systems will rely on scalable sensing infrastructures combining computer vision, thermal imaging, and machine learning. Rather than attaching sensors to each bird, such systems aim to extract individual-level information from shared sensing platforms capable of monitoring thousands of animals simultaneously, thereby enabling improved welfare monitoring, early detection of stress, and more precise environmental management.

Seeing the Unseen: AI-Powered Detection of Feeding, Drinking, and Resting Behaviors in Broiler Production

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Artificial intelligence powered by computer vision is opening new possibilities for behavior monitoring in poultry meat production. We present a computer vision-based framework for automated detection of feeding, drinking, and resting behaviors in commercial broiler systems. Our research was conducted at a large-scale broiler farm in eastern Hungary, where 21,000 Ross 308 broiler chickens were raised per fattening cycle in each poultry house, each with a floor area of 1,400 m². A total of 4 cameras were used for data collection, mounted on the ceiling and arranged in 2 rows, with 2 cameras in each row. Validation used Birdwatcher video data processed at one frame per second, generating nearly 10,000 images, from which representative samples were manually annotated in Roboflow. A YOLOv12 object detection pipeline, integrated into Google Colab and deployable locally, detects birds and assigns behaviors through dynamically defined feeder-drinker regions. Under commercial conditions, a representative model achieved 95.7% mAP@50, 93.6% precision, and 96.0% recall. A Python application extracts time-stamped events to support downstream analytics, rolling-window prediction, anomaly detection, and early welfare alerts across poultry systems.

Investigation on chickens' escape behaviour in the free-range area related to the presence of birds of prey

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Free-range systems enable laying hens to perform their full range of natural behaviour, thus improving animal welfare. Nevertheless, there are also negative aspects associated with this type of husbandry. For instance, the potential access for predators represents a major risk factor for the welfare and health of chickens using the free-range area. Chickens exhibit different escape reactions depending on the type of predator, which have so far been studied mainly under laboratory conditions. This study investigates chickens' behaviour and vocalisation in free-range when exposed to the presence of a bird of prey. The generated findings can be used for developing deterrent methods, for instance. Data were collected on a farm with a mobile housing system and one flock (990 hens, 20 roosters) with daily access to a free-range area. Video cameras and microphones recorded chickens' behaviour and vocalization in the area in the direct proximity of 11.3 m maximum to the mobile house continuously over three months (Daily from 9 am – 7 pm). Behavioural observations included the identification of the animal that first perceived the predator (first animal) and its reaction. The perception point was examined, which reflects the time point at which the predator was detected, and the following behaviour was differentiated in freezing (in the open or closed area), moving forward (at normal or increased speed) and flapping wings (while running). Additionally, chickens' vocalisation, specifically typical warning calls, and the corresponding behaviour of the flock were analysed. So far, data from five consecutive days were evaluated resulting in 63 situations in which a predatory bird was visible. The corresponding behaviour of the animals was analysed. Preliminary results showed that the first animal was a rooster in 57.1% of the situations observed, while in 31.7% it was a hen, and in 11.1% of cases it could not be clearly identified. Thus, male reactions dominated. In 74.6% of the situations, no vocalisations, respectively warning calls, were recorded. Furthermore, the results showed that after perceiving the predator, the first animal exhibited freezing in 100.0%, most often followed by movement at normal speed (63.5%) or wing flapping (17.5%). Regarding the behaviour of the flock, no consistent behaviour patterns similar to this of the first animal were observed. Nevertheless, it was apparent that most of the animals continued to move at normal speed (71.4%) when the first animal perceived the predator, while 30.2% showed freezing as well. The following behavioural patterns of the flock were variable or occurred in parallel. To date, the results indicate that the combination of behavioural and vocal analysis offers new insights and is valuable to develop potential effective deterrent methods for birds of prey. The evaluations are still being continued, and further investigation will be conducted to include flock without roosters and with other genetics. This research is funded by the Lower Saxony Ministry of Science and Culture and the Volkswagen Foundation as part of the zukunft.niedersachsen program within the ZERN project.

Automated Poultry Behavior Monitoring via Hybrid Multi-Object Tracking and Learned Visual Descriptors

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Animal welfare has become a fundamental pillar in modern poultry production, necessitating objective and scalable monitoring methods to ensure health and productivity. This study presents the design and implementation of an automated monitoring system using advanced computer vision techniques to track and analyze chicken behavior. The methodology, utilizes the YOLOv8 architecture for the robust detection and segmentation of animals in high-density environments. To address the complexities of individual tracking, the project evaluates state-of-the-art Multi-Object Tracking (MOT) algorithms, such as BoT-SORT and ByteTrack, which achieved a high precision rate of 99.7% in Multiple Object Tracking Accuracy (MOTA). A significant contribution of this research is the development of a hybrid tracking solution based on a library centered on motion prediction. To enhance identity preservation, the system integrates two distinct approaches for visual descriptor extraction. First, it employs HoG (Histogram of Oriented Gradients) to capture manual features related to texture and orientation. Second, the study implements a convolutional Auto-Encoder trained specifically on poultry image crops. This unsupervised deep learning model learns a latent space (embeddings) that captures the unique visual characteristics of the animals. Data association between consecutive frames is then solved using the Hungarian algorithm, optimizing the balance between tracking stability and computational overhead. Experimental results demonstrate the system's capability to generate detailed trajectory maps and spatial activity heatmaps, which identified non-homogeneous movement patterns within the enclosure. The Auto-Encoder approach proved to be a viable and flexible alternative to manual descriptors, yielding competitive performance in maintaining identity consistency. Finally, the study proposes future research lines involving generative AI models, such as Stable Diffusion and SAM v3, to automate data labeling and enhance the detection of complex positive behavioral events like pecking, preening and dustbathing.

From climate projections to building adaptation: a decision-support tool for heat stress in French poultry farming

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Heat stress is a growing threat to broiler production in France, as rising temperatures reduce feed intake, impair growth performance and increase mortality risk. Quantifying future thermal exposure at the farm level is therefore essential for guiding adaptation investments. We present an interactive decision-support tool that combines an ensemble of 10 regional climate models (following TRACC scenario) with a building-level thermal model to map projected heat stress indicators across metropolitan France at département resolution, from the historical period through 2099. Outdoor Temperature-Humidity Index (THI) is computed daily from maximum temperature and humidity drawn from the DRIAS climate portal, at 8 km resolution across three 30-year periods: historical (1976–2005), near future (2030–2060) and far future (2070–2099). Seven annual indicators are derived per département: exceedance days above THI thresholds of 66, 73 and 79, corresponding respectively to mild, severe, and very severe stress; annual maximum and summer-average THI (June–September); and two body weight gain loss metrics based on a −2.1 %/°C linear relationship above 21 °C, expressed as both daily-episode and annualised values. Ensemble uncertainty is communicated as median, interquartile range and full model spread, both at pixel level on spatial maps and along département-level time series spanning the full 1976–2099 period. A steady-state enthalpy and moisture balance extends the analysis indoors, solving a quadratic equation for indoor temperature as a function of outdoor conditions, ventilation rate, animal metabolic heat production, stocking density and optional evaporative misting. Indoor specific humidity is resolved from a concurrent water vapour balance accounting for animal latent heat, ventilation renewal and misting input. A physical feasibility check warns users when the configured misting rate would saturate indoor air beyond an adjustable relative humidity threshold, a situation where evaporative cooling becomes ineffective. All seven indicators are recomputed on-the-fly for any combination of building parameters, enabling direct comparison of outdoor and indoor thermal conditions. Ensemble projections show a consistent and substantial increase in heat stress indicators across all French regions and periods. Indoor modeling reveals that building configuration and misting can partially offset outdoor heat stress, though this benefit varies with local climate and animal density. Designed for direct use by farmers, technical advisors and policy makers, the framework links probabilistic climate projections to building-level adaptation scenarios in an accessible visual interface.

Direct monitoring of Salmonella typhimurium using smartphone CL imaging

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Salmonella typhimurium, a primary causative agent of enteric diseases, poses a persistent global health threat, particularly in resource-limited settings that require rapid, sensitive, and cost-effective diagnostics. Traditional diagnostic methods, such as bacterial culture and serology, are hindered by lengthy processing times, low sensitivity, and the need for specialized laboratory infrastructure. Addressing these limitations, this study presents a novel, label-free chemiluminescence (CL) gel-imaging platform for the specific detection of S. typhimurium based on aptamer-mediated signal inhibition. The bioassay utilizes agarose gels functionalized with a high-affinity anti-S. typhimurium aptamer and an intermediate metal catalyst. A luminol-H□O_2-metal ion system generates uniform light emission across the gel matrix in seconds and is stable for 10 minutes of illumination. Hence, upon sample introduction, the specific binding of S. typhimurium to the immobilized aptamers induces steric hindrance, creating distinct signal inhibition zones that are easily captured and quantified using a smartphone camera. Under optimal conditions, this point-of-care platform achieved a limit of detection of 10 CFU/mL in 1 hour and a linear dynamic range of 10_1–10_5 CFU/mL. Furthermore, the assay demonstrated >90% specificity against a broad panel of cross-reactive species, including Escherichia coli, Staphylococcus aureus, Bacillus cereus, and five other Salmonella serovars. Overall, this aptamer-driven, smartphone-readable CL-gel approach offers a robust, on-site diagnostic tool for rapid pathogen screening, with significant potential for adaptation to other enteric threats.

Dual-Mode Portable Biosensing Platform for Rapid and Sensitive Detection of Salmonella typhimurium

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The rapid increase in food contamination and associated illnesses necessitates the development of effective pre-detection strategies for microbial pathogens. In this study, we present a dual-mode biosensing platform for the efficient and reliable detection of Salmonella typhimurium (S. typhimurium) in foods. As a major contributor to enteric fever and typhoid, the detection of S. typhimurium requires constant monitoring due to its significant public health impact. Our approach depicts dual-mode detection, a reflectance-based optical biosensor using an indirect antibody assay combined with biochemical signal amplification through horseradish peroxidase (HRP) enzyme activity and photothermal imaging of the enzyme activity. The insoluble reaction products generated by HRP activity and infiltration into the porous silicon matrix alter the reflectance spectra proportionally to the bacterial concentration. Additionally, the enzyme activity was photothermally detected via TMB (3,3′,5,5′-Tetramethylbenzidine) catalysis, with reaction temperature (DT, °C) varying by logarithmic concentration. The specificity and selectivity of the anti-Salmonella antibody were validated against individual and mixed other bacterial strains. Under optimized conditions, the sensor achieved a limit of detection (LOD) of 3 CFU/mL (R_2= 0.995) and demonstrated a linear response in the range of 10_0 to 10_4 CFU/mL using the reflectance method. Additionally, the sensing platform was tested with spiked food samples, including chicken, tahina, and chocolate, exhibiting good recovery, reproducibility, and specificity. This biosensing strategy demonstrates significant potential for field-based applications, offering high sensitivity, a wide dynamic range, and rapid detection capabilities without requiring sample enrichment. The label-free, portable interferometric system functions independently of external light sources, further enhancing its suitability for on-site deployment. Importantly, this proof-of-concept platform can be readily adapted for the detection of various pathogenic microorganisms within complex food matrices.

Effect of enhanced early life nutrition on gene expression profiles of key metabolic tissues in Holstein Friesian bull calves

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Improved nutritional status in calves during the early life period has been shown to induce positive latent effects for many economically important traits, including improved lifetime growth and carcass composition. The liver has a central role in intermediary metabolism and therefore is likely to mediate the effect of improved nutrition. However, knowledge of the role of the liver towards this effect in young calves is currently lacking. The objective of this study was to evaluate the transcriptomic response of liver tissue of bull calves offered either a high or moderate plane of nutrition from 2 to 12 weeks of life. Thirty Holstein-Friesian bull calves (mean age: 17.5 days; mean weight 48.8kg) were assigned to either a high (HI; n=15) or moderate (MOD; n=15) energy dietary treatment group. Calves in the HI group were individually offered 10L of milk replacer, ad libitum concentrate and 500 g/day of hay, whilst those in the MOD group were offered 4L of milk replacer, 500 g of concentrate and 500 g hay per day. All calves were euthanised at 12 weeks of age and liver tissue samples harvested from each calf. Tissue samples were subsequently used for global transcriptomic analysis. RNA-sequencing data were analysed bioinformatically for the determination of genes differentially affected by early life plane of nutrition. Additionally, the relationship between differentially expressed genes and markers of differential growth were further established through a co-regulatory network analysis. Phenotypic markers of differential growth included average daily gain, liver weight, liveweight, milk replacer and concentrate intake as well as systemic concentrations of metabolic hormones (IGF-1, insulin) and glucose. Following differential feeding up to 12 weeks of age, HI calves achieved a growth rate of 0.88 kg/day, whilst MOD calves grew at 0.58 kg/day ($P < 0.001$). In total 174 genes were differentially expressed (adj. P-value <0.1; fold change > 1.5) in the liver between HI and MOD calves (100 genes down-regulated and 74 up-regulated in HI compared to MOD). Pathway analysis revealed enrichment (adj. P-value <0.05) of metabolic pathways, biosynthesis of amino acids and steroid hormone biosynthesis as a consequence of improved nutritional status. Co-regulatory network analysis of differentially expressed genes with phenotypic growth measurements highlighted a positive relationship between the RHOJ gene, which is involved in vascularisation, with both concentrate intake and liveweight, whilst the NTS gene, involved in fat metabolism, was positively related to glucose concentrations. Additionally, IGF1 gene expression was positively connected to growth rate, liver weight and systemic concentrations of IGF-1. Enhanced early life nutritional status contributed to altered metabolic and steroid hormone processes within the liver tissue, which may lead to subsequent improved animal performance. This study highlights an effect of enhanced nutritional status during early life on the transcriptional control of liver tissue in calves. Results from this study can be further utilised to augment early life nutritional management in cattle with potential latent benefits for overall lifetime performance. This study was funded by Science Foundation Ireland (16/IA/4474).

The first feeding matters: effects of passive immunity transfer on growth performance in dairy-beef calves under practical field conditions

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Adequate colostrum intake is essential for passive immunity transfer in neonatal calves and may influence long-term productivity. While this relationship has been widely studied in dairy replacement calves, less information is available for dairy-beef production systems under practical farm conditions. The objective of this field study was therefore to investigate the association between passive immunity transfer and growth performance of dairy-beef calves raised under practical management conditions, including a relatively restrictive milk feeding regime. The study was conducted on a commercial dairy farm and included 43 male HF-calves raised for veal. Colostrum management parameters, including colostrum volume, timing of first feeding and colostrum quality were recorded. Colostrum quality was assessed using Brix refractometry and serum samples were collected between d 2 and 7 of life to determine immunoglobulin G (IgG) concentrations using ELISA. Passive immunity transfer was evaluated using established thresholds and calves were categorized into groups with adequate (≥18 g/L) or insufficient (<18 g/L) serum IgG concentrations. After the colostrum phase calves received milk replacer twice daily (2 × 2.5–3 L; 113 g powder/L) reflecting a relatively restrictive feeding regime compared with current recommendations for intensive calf rearing. Growth performance was monitored through weighing during the rearing period and by recording carcass weight at slaughter at approximately 7.5 months of age (mean age 236 days). Data were analysed using linear regression models and mixed models including birth weight as a covariate. Overall, 37% of calves showed insufficient passive immunity transfer (<18 g/L serum IgG). Serum Brix values were strongly correlated with serum IgG concentrations (r^2=0.61), confirming their suitability as a practical on-farm monitoring tool for passive immunity status. During the first weeks of life, average daily weight gains were relatively low (approximately 0.3–0.4 kg/day), reflecting the restrictive feeding intensity on the farm. Nevertheless, calves with adequate passive immunity transfer exhibited consistently higher growth than calves with insufficient IgG concentrations. At approximately 33 d of age, adequately supplied calves already showed higher body weights (52.9 vs. 49.5 kg) and higher average daily gains. These differences persisted throughout the fattening period. At slaughter, calves with adequate IgG concentrations reached significantly higher carcass weights (150 vs. 134 kg; $P<0.05$). Correspondingly, average daily gains until slaughter were higher in adequately supplied calves (1.04 vs. 0.91 kg/day). Health parameters recorded on farm did not differ significantly between groups, which may partly reflect limitations of routine health monitoring under field conditions. However, the clear differences in growth performance highlight the importance of early-life immune status for long-term productivity in dairy-beef systems. These results indicate that adequate passive immunity transfer can partially compensate for suboptimal feeding conditions during early calf rearing. Under practical production conditions, early colostrum management therefore represents a key factor influencing long-term growth performance in dairy-beef systems. Routine monitoring of colostrum quality and passive immunity transfer using simple on-farm tools such as Brix refractometry may help identify management deficits early and improve both animal health and production efficiency in dairy-beef production systems.

Effect of post-weaning or pre-finishing concentrate supplementation at pasture on growth and carcass characteristics of early-maturing dairy-beef steers

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In Irish pasture-based dairy-beef systems, early-maturing, spring-born steers are typically finished over 25 months during a third grazing season, whereas current research blueprint systems achieve finishing at 22-24 months during the second indoor winter. Reducing finishing age to 19 months, prior to a second winter housing, can improve profitability and sustainability; however, achieving adequate subcutaneous carcass fat remains a primary challenge for low-input, early-finish grass-based production systems. A high plane of nutrition during early life (3–21 weeks of age) increases adipose hyperplasia and hypertrophy in calves harvested at 5 months of age. Improved early-life nutrition may promote adipogenesis and feed efficiency, potentially predisposing cattle to a greater carcass fat score when finished at an earlier developmental stage. The aim of this study was to determine the residual effect of post-weaning or pre-finishing plane of nutrition at pasture and finishing age on growth and carcass characteristics of early-maturing dairy-beef steers. Spring-born Aberdeen-Angus × Holstein-Friesian male (n=100) were assigned to one of five production systems following a common indoor calf rearing period (n=20/system). (Control): 22-month finishing age following indoor winter finishing on grass silage + 375 kg concentrate DM (Total lifetime concentrate, 750 kg DM). S2-S5: 19-month finishing age at the end of the second grazing season; S2 & S3: Received high (375 kg) and medium (185 kg) concentrate DM, respectively, during the first grazing season, to exploit early-life feed efficiency, and no concentrates pre-finishing (Total concentrate, 750 kg and 565 kg DM, respectively). S4: Pasture + 375 kg concentrate DM for 90 days pre-finishing (Total concentrate, 750 kg DM). S5: Pasture + zero concentrates pre-finishing (Total concentrate, 375 kg DM). Live weight was recorded regularly and post-slaughter, carcass data were collected. Data were statistically analysed using mixed models. During the first grazing season, average daily live weight gain was greater ($P < 0.05$) for S2 and S3 than S1, S4 and S5, resulting in S2 and S3 being heavier at housing ($P < 0.05$). At the start of the second grazing season, live weight remained heaviest for S2, lightest for S1, S4 and S5 ($P < 0.05$), however S3 was intermediate ($P > 0.05$). During the second grazing season live weight gain was greater ($P < 0.05$) for S4 (supplemented pre-finishing) compared to all other systems (un-supplemented). Carcass weight was heavier ($P < 0.05$) for S1 (22 months) compared to the 19-month systems, which did not differ. No differences were observed in carcass conformation or fat scores between production systems ($P > 0.05$). Although the additional three months of development for S1 yielded the heaviest carcasses, the 19-month systems achieved commercially acceptable fat scores (mean ≥6.0 on a 15-point scale). Feeding half of the allocated 'finishing' concentrate budget (185 vs. 375 kg DM) in the first grazing season (S3), compared to all of the allowance (375 kg DM) in the first grazing season (S2) or pre-finishing during the second grazing season (S4), effectively supported lifetime carcass gain without compromising carcass specifications, while reducing concentrate input and obviating a second winter housing period. The economic and environmental implications of reallocating lifetime concentrate budgets and reducing finishing age merit further assessment.

Effects of nutrition and methane-suppressing feed additives on plasma metabolites of pre-weaned replacement Holstein heifers

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Variations in nutrient intake in pre-weaned calves have shown to activate epigenetic mechanisms that affected the long-term animal performance. Feeding methane suppressing feed additives to pre-weaned calves can affect the rumen microbiota by producing less CH4; however, this may also result in ruminal fermentation changes that compromises nutrients intake and animal performance. Feeding calcium peroxide (CaO2) has shown to reduce up to 29% methane production in finishing beef cattle; however, it has also shown reduction in nutrient digestibility and volatile fatty acid production. The aim of this study was to evaluate the effects of pre-weaning milk intake and methane-suppressing feed additives (MSFA) in the starter concentrate on the early metabolic development of replacement heifers. Thirty-six replacement Holstein calves were randomly arranged in a 2 x 2 Latin square experimental design. Calves were fed milk replacer (MR) at either 4 or 8 L for 63 days (step-weaned from days 42-63) and supplemented with a starter concentrate containing either Calcium carbonate (control) or CaO2 (MSFA) from days 5 to 83. Heparin plasma samples were taken at day 63 of age and frozen at -80°C until metabolomic analysis. Ultrahigh Performance Liquid Chromatography-Tandem Mass Spectroscopy was used to analyse plasma metabolites. Following imputation of missing values, and log transformation, a two-way ANOVA was used to identify biochemicals that differed significantly between experimental groups. Biochemicals that achieved statistical significance of $p \leq 0.05$ were analysed. Results showed that the intake of CaO2 affected ($P < 0.05$) the microbial activity by decreasing some metabolites of secondary bile acid sub pathways and increasing those in the benzoate sub pathway. Metabolites related to energy metabolism showed that calves drinking 8L MR but consuming CaO2 fold decreased ($P < 0.05$) glucose, lyxonate, fructose and mannose in plasma by 0.87, 0.64, 0.84 and 0.75, respectively, when compared to control calves. Dicarboxylic fatty acids metabolites were elevated ($P<0.05$) in the plasma of all calves fed CaO2 when compared to control calves. Additionally, calves fed 4L MR and CaO2 had a 1.51-fold increase ($P<0.05$) in 3-hydroxybutyrate. For the protein catabolism, all calves consuming 8L MR, especially calves fed CaO2 , decreased ($P < 0.05$) plasma metabolites in sub pathways such as: amino acid metabolism, branch chain ammino acids, methionine metabolism and dipeptides. Calves fed 8L MR and CaO2 not only showed reduction ($P < 0.05$) in metabolites corresponding to metabolic cycles such as glutamate and methionine, but also in gamma-glutamyl ammino acids and vitamins C, B5 and A when compared to their counterpart consuming 4L MR and CaO2. It is concluded that the intake of CaO2 affected the metabolism of the rumen microbial ecosystem by increasing some metabolite derived from microbial activity in the host's plasma. In the calves, adding CaO2 to the starter concentrate reduced the metabolism of carbohydrates, whilst favouring the metabolism of fats. Additionally, the ingestion of 8L MR decreased the catabolism of proteins and ammino acids, and oxidative metabolites, however, those calves consuming CaO2 showed lower concentrations of vitamins C, B5 and A in plasma. Future work might involve the analysis of additional time points pre- and post-treatment to determine the impact of milk replacer intake and calcium peroxide supplementation in the long-term of replacement heifers.

Effect of dietary supplementation in Rubia Gallega calves assessed by ultrasound measurements

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Dietary supplementation with essential oils derived from hydrolysed R. communis oilseed (Bovino Plus ®) was evaluated as a nutritional strategy to modulate dorsal subcutaneous fat thickness (SCF12) and Longissimus muscle area (ULMA) at the 12th-13th rib interface. Twenty-four Rubia Gallega male calves from Mabegondo Agricultural Research Centre herd were randomly assigned to one of three treatment groups for 90 days: control (G0), 10 mL/animal/day of essential oils supplement (G1) or 20 mL/animal/day of essential oils supplement (G2). Animals were fed indoors with concentrate and hay ad libitum. The intake of concentrate was calculated daily for each group as the difference between the amount offered and the amount refused (mean daily intake 6.08 kg DM/calve/day). Male calves were weighed every 15 days from weaning to slaughter to determine average daily gain (ADG). In addition, thoracic perimeter (TP) was measured using a tape measure. The 24 male calves were subjected to two ultrasound exams: one at weaning and another immediately prior to slaughter. Ultrasound measurements were performed using an EXAPAD MINI device (Humeco) to assess SCF12 and ULMA in transverse section. Images were obtained using a 2.5 MHz linear transducer integrated into an Exapad Mini unit (Humeco). The imaging parameters used were as follows: depth 160 mm, frequency 2.5 MHz, focal zone 50 mm, and gain 60 dB. The images were subsequently analysed using MeatQtex software (Humeco). Animals were slaughtered at 312 days of age, with mean slaughter live weights (SLW) of 449.25 kg (G0), 437.25 kg (G1), and 465.87 kg (G2), and a mean carcass weight (CW) of 250 kg (G0), 243 kg (G1) and 272 kg (G2). Carcasses were classified according to the EUROP system as R+2+ for G0, U–2+ for G1, and U2+ for G2. All statistical analyses were performed using R Commander for Windows, version 4.3.2. No significant differences were found between treatment groups for Longissimus muscle area between the 12th-13th rib interface at weaning (ULMAw) and at slaughter (ULMAs). ULMAs values were significantly greater than ULMAw in all groups, with no significant differences among groups (G0: ULMAw 66.68 ± 1.71 vs. ULMAs 75.50 ± 6.38; G1: ULMAw 67.68 ± 5.38 vs. ULMAs 76.99 ± 4.96; G2: ULMAw 71.41 ± 4.85 vs. ULMAs 82.34 ± 4.40, $P < 0.001$). No significant differences were observed among treatment groups for subcutaneous fat thickness at the level of the 12th -13th rib interface at weaning (UFATw) and at slaughter (UFATs). UFATs values were significantly higher than UFATw in all groups, with no significant differences among groups (G0: UFATw 2.89 ± 0.19 vs. UFATs 3.68 ± 0.31; G1: UFATw 2.65 ± 0.21 vs. UFATs 3.38 ± 0.11; G2: UFATw 2.70 ± 0.19 vs. UFATs 3.69 ± 0.41, $P < 0.001$). ULMAs showed moderate to high positive Pearson´s correlations (95% confidence intervale, $P < 0.01$) with TP at slaughter ($r = 0.46$), CW ($r = 0.54$), dressing percentage ($r = 0.60$) and carcass conformation ($r = 0.67$), indicating that ULMAs values are a good indicator of overall muscular development in Rubia Gallega male calves. Moderate correlations between ULMAs and ADG ($r = 0.35$), between ULMAs and SLW ($r = 0.44$) were observed. In conclusion, R. communis oilseed supplementation did not modify dorsal subcutaneous fat thickness and Longissimus muscle area at the 12th -13th rib by ultrasound measurements in Rubia Gallega male calves. However, ultrasound Longissimus muscle area was a practical and reliable indicator of carcass weight, dressing percentage and carcass conformation, as shown by high correlation values.

A survey of dairy-beef calf management practices pre- and post-weaning, and post-turnout to pasture on Irish commercial farms

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The expansion of dairy cow numbers has led to an increase in dairy calves available for beef production in Ireland; however, information on dairy-beef calf management practices on Irish commercial farms remains limited. The aim of this study was to describe the management of Irish dairy-beef calves pre- and post-weaning, and post-turnout to pasture. A survey of 43 farms geographically distributed across Ireland was conducted using a combination of face-to-face and online questionnaires between February and August 2025. Farms were recruited through the online and farming media advertising, Teagasc farm advisory service, and public farming events. Participation in the survey was voluntary. Most farms reared both male and female calves (69%, 25/36) and purchased calves at 2-4 weeks of age (92%, 33/36) directly from multiple (median: 4) dairy farms (72%, 26/36). Predominant breeds of calves purchased were Aberdeen Angus (81%, 29/36), Hereford (58%, 21/36), and Holstein-Friesian (58%, 21/36). Calf health (79%, 27/34), price (62%, 21/34), and beef genetic merit (commercial beef value; 38%, 13/34) were the main purchase criteria. Upon arrival, access to water and forage (100%, 36/36), electrolytes (31%, 11/36), and calf milk replacer (67%, 24/36) was provided immediately. On all farms (36/36), calves were accommodated in group pens, with group sizes of ≤10 (48%, 16/33), >10-20 (21%, 7/33), >20-30 (21%, 7/33), and >30 (9%, 3/33) calves. Multiple criteria were used for grouping, including purchase batch (53%, 18/34), bodyweight (35%, 12/34), and age (21%, 7/34). Almost all farms (97%, 35/36) practiced calf vaccination (bovine respiratory (86%, 30/35) and clostridial (60%, 21/36) diseases), and of those, 66% (23/35) administered vaccines within 7 days post-arrival. Most farms fed calf milk replacer twice daily (81%, 29/36), with a daily milk volume of 5-6 L (83%, 30/36) at total solids concentrations of 12.5-15% (76%, 22/29). Pre-weaning, all farms (36/36) offered concentrate (mostly ad libitum; 83%, 30/36) and forage (all ad libitum). Gradual weaning was practiced on all farms (36/36), with half of the farms (17/34) weaning calves over at least 7 days. Multiple criteria were used for weaning, with age (60%, 21/35), concentrate intake (60%, 21/35), and bodyweight (49%, 17/35) being most prevalent. Predominant weaning target included age of 10-12 weeks (55%, 11/20), concentrate intake of 1.5-2 kg/day (86%, 18/21), and a bodyweight of 90-100 kg (71%, 10/14). Most farms weaned calves indoors (74%, 29/39), and among these, 93% (27/29) retained them indoors for 1-4 weeks before turnout to pasture. Calf turnout mostly occurred in multiple batches (75%, 30/40) from early March to early June (median: late April). All farms (40/40) continued concentrate feeding post-turnout, with 73% (29/40) offering 1.5-2 kg/day. Almost two-thirds of the farms (24/38) offered pelleted concentrate, with the remainder offering coarse ration (34%, 13/38), at pasture. On 22 farms (55%), concentrate feeding was continued throughout the entire grazing season. Most farms operated rotational grazing systems (83%, 33/40) and calves grazed separate pasture areas from adult cattle (69%, 27/39). A leader-follower grazing regime was practiced on 15% (6/39) of farms. This study identified variation in management practices of dairy-beef calves on Irish commercial farms. These findings may help to inform future research and to guide targeted knowledge-transfer initiatives for dairy-beef farmers.

Umbilical Cord Blood Profiling at Birth as a Predictor of Early Growth in Piglets

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Newborn piglets face substantial physiological challenges immediately after birth, including thermal stress, adaptation to extrauterine respiration, and high metabolic demands. Their survival and subsequent growth largely depend on their ability to respond effectively to these early-life stressors. Previous research suggests that physiological and behavioural differences between pigs with divergent early growth are marked by the suckling period and associate with persistent physiological alterations later in life. The objective of this study was to identify and evaluate non-invasive physiological parameters measurable at birth that could predict average daily gain (ADG) during the first week of life. The study included 40 randomly selected liveborn (Pietrain × (Landrace × Large White)) piglets from seven multiparous sows within a single farrowing batch on a 600-sow commercial farm operating with a 4-week batch management system. Umbilical cord blood was collected within ten minutes of birth, prior to colostrum intake, using a syringe and immediately analysed with a CG8+ cartridge on a portable i-STAT® analyser. Measured parameters included blood gases (partial carbon dioxide (pCO2) and partial oxygen), electrolytes (sodium and potassium), pH, glucose (Glu), ionized calcium, and haematocrit. Calculated values for total CO_2 (tCO2), bicarbonate (HCO^{3-}), base excess (BE), oxygen saturation, and haemoglobin were also obtained. Piglet body weight was recorded at birth, 24 hours, and 7 days to calculate ADG. Univariate linear regression analyses were performed to assess associations between umbilical cord blood parameters and weight gain. First-day weight gain (0–1 WG) was positively correlated with Glu ($r = 0.358$, $p = 0.031$) and first-week ADG ($r = 0.438$, $p = 0.012$). ADG during the first week was negatively correlated with BE ($r = –0.370$, $p = 0.043$), HCO^{3-} ($r = –0.393$, $p = 0.029$), and tCO2 ($r = –0.396$, $p = 0.030$). Notably, Glu was negatively correlated with BE ($r = –0.629$, $p < 0.001$), HCO^{3-} ($r = –0.585$, $p < 0.001$), and tCO2 ($r = –0.574$, $p < 0.001$). The positive correlation between 0–1 WG and Glu suggests that piglets with greater immediate energy availability at birth are better prepared for the required postnatal transitions. These piglets may exhibit greater vitality, facilitating earlier access to the udder and increased colostrum intake, which is reflected in higher weight gain within the first 24 hours. However, Glu was not significantly correlated with 0–7 ADG, suggesting that factors beyond initial colostrum intake also contribute to early growth. The negative associations between 0–7 ADG and multiple acid–base parameters provided by the i-STAT® analyser (BE, HCO^{3-}, and tCO2) indicate that acid–base status plays a significant role in early postnatal growth. The negative correlations between Glu and these parameters further suggests a metabolic advantage at birth for piglets with lower levels. Increases in HCO^{3-}, tCO2, and BE, calculated from pCO2 and pH, may reflect a transient episode of intrapartum hypoxia and associated metabolic stress, which could compromise the piglet's ability to adapt effectively to the competitive and demanding postnatal environment. This study demonstrates that specific umbilical cord blood parameters measured immediately after birth are significantly associated with early postnatal growth in piglets. Acid–base balance parameters and glucose concentration may serve as practical early biomarkers of neonatal performance, offering valuable tools for identifying piglets at risk of suboptimal growth.

Effects of maternal undernutrition on cardiac mitochondrial DNA (mtDNA) abundance and the cardiac transcriptome in Japanese Black fetuses

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Maternal nutrition during gestation plays a critical role in fetal organ development and long-term physiological function. In cattle, prenatal undernutrition has been associated with impaired postnatal growth and increased susceptibility to metabolic and cardiovascular disorders. However, the molecular mechanisms underlying fetal cardiac adaptation to maternal undernutrition remain unclear in cattle. This study aimed to investigate the effects of maternal undernutrition on mitochondrial DNA (mtDNA) abundance and global gene expression profiles in the fetal heart of Japanese Black (Wagyu) fetuses. Multiparous Japanese Black cows were randomly assigned from conception to 8.5 months of gestation to either a high-nutrition group (HN; 120% of nutritional requirements, n = 6) or a low-nutrition group (LN; 60%, n = 6). Fetuses were collected near term, and left ventricular cardiac tissue was obtained for molecular analyses. Total DNA and RNA were extracted from each sample. Mitochondrial DNA copy number was quantified by qPCR using mitochondrial-encoded genes normalized to nuclear genomic DNA. Transcriptomic profiling was performed using RNA sequencing (RNA-seq), followed by differential gene expression and pathway enrichment analyses. The mtDNA copy number in fetal cardiac tissue was significantly lower ($P < 0.05$) in LN compared with HN, indicating reduced mitochondrial abundance under maternal undernutrition. RNA-seq analysis identified 1,847 differentially expressed genes (DEGs) between LN and HN using the criteria $|\log_2 \text{fold change}| > 2$ and $q < 0.05$. Pathway analysis demonstrated that genes involved in mitochondrial oxidative phosphorylation, the tricarboxylic acid cycle, and fatty acid β-oxidation were predominantly downregulated in LN, whereas genes associated with extracellular matrix organization, focal adhesion, and cellular stress responses were relatively upregulated in LN compared with HN. In conclusion, maternal undernutrition during gestation reduces mitochondrial content and induces coordinated transcriptomic alterations in the hearts in Japanese Black fetuses, characterized by suppression of energy metabolism–related pathways in the low-nutrition group.

Effects of maternal undernutrition on binucleate cell abundance and gene expression in the placenta of Wagyu (Japanese Black cattle)

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Maternal nutrition during pregnancy may influence fetal development through placental adaptation, but the nutritional responsiveness of placental phenotypes and their molecular correlates in beef cattle remain unclear. This study investigated whether sustained maternal nutritional restriction from pre-gestation through late pregnancy alters binucleated cell (BNC) abundance in placenta of Wagyu and whether corresponding differences in gene expression are detectable by RNA sequencing (RNA-seq). Primiparous Japanese Black cattle (n=12) were assigned to either a high-nutrition group (HN; 120% of nutritional requirements, n=6) or a low-nutrition group (LN; 60%, n=6) from pre-gestation until gestation day 260. Placentae were collected via cesarean section at 260 days of gestation. Hematoxylin and eosin staining was performed to morphologically identify BNCs. BNC abundance was quantified as the total number of BNCs counted from 10 microscopic fields (×400) per sample using BZ-X 1000 (KEYENCE, Osaka). Total RNA was extracted from each placenta for transcriptome analysis. Differentially expressed genes (DEGs) between groups were identified using DEGseq2 ($q < 0.05$). The BNC count was significantly larger in the HN group than in the LN group, indicating that long-term maternal nutrition influences trophoblast cell dynamics in the placenta. Differential expression analysis identified only three genes: COL6A5, KCP, and KRT17 ($q < 0.05$). These three genes are associated with structural organization of the extracellular matrix. However, the small number of detected genes suggests that gene expression changes are minor. Under the current sampling and analysis conditions, detectable transcriptome differences between nutritional groups are limited. Overall, maternal nutritional status was associated with distinct histological phenotypes, but RNA-seq detected only minor differences in gene expression. Although gene expression variability was small, changes in genes associated with extracellular matrix structural alterations may contribute to alterations in binuclear cell numbers.

Tributyrin supplementation reduces diarrhea frequency and severity in preweaning calves

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Neonatal calf diarrhea is one of the biggest health challenges in dairy cattle herds, causing substantial losses. In this regard, butyrate was shown to promote intestinal development, improve gut health and reduce the diarrhea incidence in young animals. This study investigated the effects of tributyrin as a butyrate source in milk replacer on diarrhea occurrence, growth performance, metabolic status and fecal microbial load in female dairy calves. Fifty female Holstein calves averaging 43.0±1.53 kg body weight (BW) at birth were divided in two experimental groups and fed a diet without (CON) or with 4.0 g/kg DM of tributyrin (TRIB; SpeoCare® T60, 60% tributyrin, Evonik Operations GmbH) mixed in a commercial milk replacer until weaning (~80 days of life). The calves were housed in individual pens during the first 3 weeks of life, in double pens the following 2 weeks (week 4-5), and collective pens (maximum 14 animals/pen) until the end of the trial (~150 days of life). The fecal consistency was evaluated daily until week 6 of life, with fecal scores from 1 to 4. Animals with fecal scores of 1 and 2 were defined as normal, while animals with fecal score of 3 (moderate diarrhea) and 4 (severe diarrhea) were defined diarrheic animals. Diarrhea frequency was calculated with the equation: diarrhea frequency (%) = [(number of diarrheic calves × days of diarrhea) / (total number of calves × days of measurement)] × 100. Calves BW was recorded at birth, at weaning, and at the end of the trial period and used for average daily gain (ADG) calculation. From each experimental group, 10 calves were randomly selected for blood and fecal sampling. Blood was sampled at birth, weaning and end of the trial period, and analyzed for total protein, hemoglobin, urea, glucose, insulin, beta-hydroxybutyrate, and glutathione peroxidase. Fecal sampling occurred at the end of week 1, 2 and 3, at weaning and at the end of the trial period. Plate count of total bacteria, lactic acid bacteria and coliform bacteria was done on the fecal samples. For statistical analyses, the qualitative data were analyzed using Pearson's square Qi test, while the quantitative data were analyzed based on a linear model using treatment and period (time) and their interaction as fixed effects. The initial measurements at birth were included as covariates. Least square means were compared using Tukey's test. Significance was declared when $P \leq 0.05$ and tendency with $0.05 < P \leq 0.10$. Supplementation with TRIB during the preweaning period significantly decreased the diarrhea frequency during the first week (-13%, $P<0.010$). More specifically, moderate diarrhea was reduced by 7% ($P=0.053$) and severe diarrhea by 6% ($P=0.066$) when compared to CON. Starting with week 2 the moderate diarrhea was not different between the two experimental groups; nevertheless, a significantly lower severe diarrhea frequency was registered with TRIB compared to CON during week 2 (-8%, $P<0.05$). Between week 3 and week 6, diarrhea frequency presented no significant difference between the two groups. No significant differences were registered in ADG or blood metabolites throughout the study. No significant differences were registered in fecal count of total bacteria or lactic acid bacteria between the CON and the TRIB groups. However, the coliform bacteria fecal load was significantly lower in the TRIB group during the whole study period ($P<0.05$; 6.34 and 7.07 $\log_{10}$ CFU/g for TRIB and CON, respectively). In summary, supplementing tributyrin during the preweaning period improved the health status of dairy calves by reducing diarrhea frequency and fecal coliform bacteria load.

Effect of early life high nutrition on hepatic metabolism, gene expression, and DNA methylation in beef cattle

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Early high nutrition potentially enhances growth and improves carcass traits of beef cattle. We hypothesized that this early nutritional effect is mediated by metabolic programming of liver, leading to modulation of endocrine and nutrient distribution. To investigate the underlying mechanisms of epigenetic modulation of gene regulation and metabolism in the liver, we examined the metabolome, transcriptome and epigenetic profiles of Japanese Black (JB; Wagyu) steers that were fed a high-nutrient milk diet in early life, using an integrated multi-omics approach. The steers were divided into two groups. The early roughage (ER) group (n = 11) was fed a standard amount of milk replacer until three months of age (mo), followed by only roughage from four to ten months. The early high overall nutrition (EHN) group (n = 12) was provided with three times the standard amount of milk replacer until three months of age, followed by a high-concentrate diet from four to ten months. From 11 to 31 months of age, all of the steers were fed only roughage. Liver samples from four EHN steers with the greatest final body weight (BW) and four ER steers with the lowest BW were used for subsequent analyses including CE-TOF/MS and LC-TOF/MS, RNA-seq, and RRBS. The body weight (BW) of EHN steers was higher than that of ER steers at 3, 10 and 31 months, as was their hot carcass weight ($p < 0.01$). EHN steers exhibited higher subcutaneous fat thickness and intramuscular fat content than ER steers ($p < 0.05$) for the carcass trait. EHN steers exhibited a liver metabolome profile with higher levels of carnitine, the precursor γ-butyrobetaine, glutamine and serine, and lower levels of acylcarnitines than ER steers. This suggests that acylcarnitine is transferred more efficiently into the mitochondria of EHN steers for accelerated β-oxidation. The results of RNA-seq and subsequent gene ontology analysis revealed that the expression of genes associated with pyruvate metabolism, gluconeogenesis, triglyceride homeostasis, lipid metabolism and the regulation of RNA polymerase activity was significantly altered. This was characterized by the upregulation of PC, APOA5, ANGPTL8, TGIF1, UHRF1 and HDAC5. Meanwhile, most of the downregulated genes were associated with the immune and inflammatory responses. Furthermore, the results of DNA methylation analysis using RRBS indicated that promoter-hypomethylated genes were associated with regulation of angiogenesis, axon guidance, regulation of mitochondrial outer membrane permeabilization, blood vessel morphogenesis, nerve system development, hepatocyte apoptotic process, chromatin remodeling, and genomic imprinting. Meanwhile, promoter-hypermethylated genes were associated with regulation of apoptotic process and regulation of cell proliferation. Although many genes with highly altered promoter methylation did not exhibit significant changes in gene expression, those involved in systemic homeostasis and energy metabolism (IGFALS, ACSF3 and ORM1) exhibited promoter region hypomethylation, which could result in increased gene expression. Collectively, EHN leads to a long-lasting metabolic shift towards high β-oxidation activity and modulates the regulation of genes involved in systemic energy metabolism in the livers of JB steers through metabolic gene imprinting.

Estimation of genetic heterogeneity of variance (environmental sensitivity) of milk yield traits and somatic cell count to predict resilience of dairy cattle

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Genetic selection offers a promising opportunity to improve resilience in dairy cattle because resilience-related traits show heritable variation. However, the lack of suitable phenotypes is a major barrier to developing genetic evaluation for resilience of dairy cattle. Variability in traits that are frequently recorded such as milk yield can be used to develop a breeding value for resilience. This study quantifies the genetic residual variability of sires based on milk yield traits and somatic cell count (SCC) and evaluates whether heterogeneity of variance at the sire level is associated with functional traits in Australian dairy cattle. Test-day data of over 760,000 Holstein (H) and 138,000 Jersey (J) cows were analysed using a Double Hierarchical Generalised Linear Model (DHGLM). In the DHGLM, mean (normal) and the dispersion (gamma) were analysed in a bi-variate mixed model including covariances between the mean and dispersion effects. We analysed three milk yield traits, fat & protein percent, fat to protein ratio and SCC to calculate sire solutions for trait dispersion. Sire solutions for dispersion of the traits were correlated with Estimated Breeding Values EBVs for functional traits as well as with exit by 180-days and exit by 180-days plus all deaths to assess their usefulness to predict resilience. Results show that the heritability of the dispersion of all the traits ranged from 0.02 (SCC and protein%) to 0.05 (fat % and fat to protein ratio). The genetic-level (sire) correlation between the mean and dispersion are higher (0.05 to 0.51) than the cow level (-0.1 to 0.34) correlations. Genetic correlation between the mean and dispersion were the highest for milk yield (MY) in H (0.51) and J (0.49). Accounting for sire by herd interaction for the mean did not affect sire solutions for dispersion; accounting for persistency of lactation, however, resulted in some re-ranking of sires for dispersion of the traits highlighting the importance of persistency on dispersion of different traits. The correlations between sire solutions for dispersion and EBVs for functional traits such as fertility, survival, etc. were low but mostly in the expected direction. For example, high sire solutions for dispersion of milk yield were associated with poorer fertility and increased mastitis incidence, and higher rate of exit by 180-days plus all deaths. Although these correlations between sire solutions for dispersion and EBVs were not strong enough to be used for predicting functional traits or developing breeding values for resilience, avoiding the use of sires with high dispersion solutions (i.e. the high sensitivity sires) may help to improve functional traits and potentially resilience. Further examination of the sire solutions for different dispersion traits showed significant differences in EBVs for functional traits between the least sensitive (bottom 25%) and most sensitive (top 25%) sires. There is interest to assess the relationship of sire solutions for dispersion traits with metabolic diseases (milk fever and ketosis) for which there is no genetic evaluation in Australia.

Deriving environmental variability indicators from herd-level milk production by calendar date for reaction norm models

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Dairy cows respond differently to changes in environmental quality, which can be characterized by both environmental level (ENV_l) and environmental variability (ENV_var). Most existing studies focus on genetic differences along ENV_l, for example, by using contemporary group estimates or daily average climatic indicators in reaction norm models. However, indicators specifically characterizing ENV_var in dairy cattle remain limited. The aim of this study was to derive an ENV_var indicator that is largely independent of ENV_l by using herd-level milk production by calendar date (HMPC) statistics. These indicators can subsequently be incorporated into reaction norm models to investigate genetic responses of traits such as resilience under variable environments. This analysis was based on data from 13 Swedish dairy herds from the network of the Gigacow project (Swedish University of Agricultural Sciences). After quality control, the dataset included 1,384,332 daily milk yield records from 3,937 lactations between 1 January 2021 and 31 December 2025. The studied herds had at least one year of milking records and a minimum of 20 daily milk yield records per date. The HMPC was derived separately for primiparous and multiparous cows. To derive ENV_l and ENV_var for individual cow-lactations, the relevant HMPC segments were obtained by aligning calendar dates with that cow's days in milk (DIM). The mean and standard deviation of HMPC were then calculated as indicators of ENV_l and ENV_var, respectively. To evaluate the relevance of ENV_l and ENV_var, individual cow-lactation DIM curves were combined with expected lactation curves derived from polynomial quantile regression models to obtain resilience traits, such as logarithm variance of deviations (LnVar) and average daily milk loss (ML_d). Associations between these resilience traits and both ENV_l and ENV_var were evaluated. The current results showed that no lactation-stage patterns were observed in HMPC, indicating that variation in HMPC largely reflected external environmental perturbations. A weak association between ENV_l and ENV_var was observed (Pearson correlation coefficient = -0.30), suggesting that the proposed ENV_var indicator is largely independent of ENV_l. Both LnVar and ML_d increased linearly with ENV_var, indicating greater milk deviations and milk loss under more variable environments. This relationship was stronger in multiparous cows than in primiparous cows (Pearson correlation coefficients of 0.87 and 0.53 for ML_d, respectively). These results demonstrate the feasibility of using the proposed ENV_var indicator to characterize environmental variability in dairy production systems. Future work will focus on investigating genetic differences in responses to ENV_var by incorporating this indicator into reaction norm models which will improve understanding of the genetic mechanisms underlying resilience to environmental variability. This work was funded by project European Partnership on Animal Health and Welfare (European Union's Horizon Europe Project 101136346 EUPAHW).

Integrating satellite environmental proxies to optimise genotype-by-environment interaction assessment for weaning weight in Namibian pasture-based beef systems

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In open rangeland cattle systems, such as those found in Namibia, there is a need for innovative environmental covariates (ECs) that extend beyond traditional metrics like temperature–humidity index (THI) to model key production factors, including forage availability and animal health stressors. Such ECs enable a more comprehensive characterization of production conditions, thereby informing breeding strategies for enhanced animal resilience to environmental pressures. This study investigated genotype-by-environment interaction (G×E) for weaning weight (WW) in four Namibian cattle breeds along post-natal conditions defined by four ECs: normalized difference vegetation index (NDVI), precipitations (PREC), surface solar radiation (SSR) and THI. The NDVI as an indicator of vegetation and forage status was contrasted to PREC, whose effects on feed availability are modulated by soil properties and other climatic factors. The SSR, given its direct impact on animals' exposed environments, was compared with THI. The ECs were derived from satellite-based data retrieved via the Google Earth Engine platform using farm coordinates and averaged over a 90-day period following each calf's birth. Pedigree-based reaction norm models were fitted separately for Bonsmara (n = 26,624), Brahman (n = 25,125), Simbra (n = 14,984), and Simmentaler (n = 9,811), to regress the genetic parameters of WW on individual EC using first-order Legendre polynomials. Reactions norms and phenotypic plasticity were assessed to identify breed-specific patterns of animal sensitivity to the ECs. Pairwise correlations between NDVI and PREC (0.70–0.79) and between SSR and THI (0.77–0.86) were moderate to high, whereas correlations between NDVI and SSR (0.03–0.26) were low, suggesting that each EC captures distinct environmental conditions. Irrespective of the breed, direct and maternal heritabilities (h^2) for WW exhibited considerable variations along all EC gradients, with NDVI and THI showing the largest variations. Estimates of h^2 increased gradually from low to higher NDVI, indicating that improved feed resources enhance response to selection and maximal genetic gain. Conversely, h^2 decreased under moderate THI and increased under both extreme heat conditions. Both direct and maternal genetic correlations (rgEC) decreased significantly, reaching negative values with environmental divergence (direct rgEC: -0.25 – 0.39; maternal rgEC: -0.68 – 0.32 at extreme EC gradients). These findings indicate the presence of G×E across all ECs, but the variation of rgEC along the gradients differed with ECs and breeds. Simmentaler and Simbra cattle exhibited the lowest rgEC at extreme EC gradients compared to Brahman and Bonsmara. Analysis of reaction norm patterns revealed low to moderate correlations between the intercept and slope of direct genetic effects (-0.2 – 0.6), confirming substantial reranking of breeding values along most ECs. Moreover, higher slope-to-intercept genetic variance ratios (> 0.5), particularly with THI and SSR in Simmentaler and Simba, suggest important contribution of environmental sensitivity to overall genetic variation in these breeds. Overall, the evaluated ECs induced notable alterations of genetic parameters in Namibian breeds. Differences in environmental sensitivity across breeds and ECs reflect varying adaptive capacities to distinct stressors and highlight that no single covariate can fully represent the complexity of G×E.

Population reaction norms of three German dairy cattle populations regarding heat stress

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Heat stress events present a challenge for global livestock production. The consequences of climate change and more extreme weather events are already becoming apparent all over the world. In general weather in Europe is more localized. Southwest Germany, in particular, comprises different natural areas, with different local climate conditions. In this context we aimed to estimate heat stress effects in southwest Germany in the three most common dairy cattle breeds: German Holstein Friesian, German Fleckvieh, and German Brown Swiss. To quantify the environmental conditions inside stables, data of installed climate loggers inside stables as part of the EIP Project KlimaFit were used. Daily milk yield measurements from automatic milking systems at 46 farms were used as animal performance of 2,538 German Holstein Friesian, 1,829 German Fleckvieh, and 815 German Brown Swiss dairy cows and the climate data from the climate loggers were used as the environmental descriptor. Daily temperature and humidity measurements were used to build a daily Temperature-Humidity-index (THI), which acts as an indicator for the daily heat stress load inside the stables. In Contrast to the common approach in most studies the THI records were not clustered into predefined groups. Instead, treated as a continuous variable using a double hinge function transformation in order to increase the resolution in the higher THI range and capture effects in this critical region. Higher order Legendre Polynomials were used in the random regression reaction norm model to describe the genetic variation in milk yield along the trajectory. The higher order Legendre polynomials were significantly above zero, confirming the presence of genetic variability of heat stress milk yield depression. The critical of the reaction norm models were slightly different between the populations. The critical value for HF was around 55, for Fleckvieh 65, and for Brown Swiss it was around 69. In all three breeds the milk yield dropped nearly linearly exceeding a population individual critical point. No greater differences were observed throughout the reaction norm between the three breeds. The results indicate that heat stress is affecting dairy cattle in southwest Germany. Furthermore, the estimated genetic variances indicate that animals differ in their genetic ability to respond to varying thermal conditions. Additive genetic effects and heritability did change along the environment THI, u-shapes were observed and were comparable between the three breeds. Future breeding should consider enhanced heat stress resilience and tolerance, which is expected to become an important issue in livestock production systems due to progressing climate change conditions.

Genetic analysis on heat tolerance of fertility traits in Canadian Holstein cows

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Adaptability to changing environmental conditions is crucial for the long-term sustainability of dairy cattle production. While the genetics of heat tolerance in milk production have been extensively studied, those of fertility traits remain less well characterized. Time from first service to conception (FSTC) reflects a cow's ability to conceive at the first insemination and is used in Canadian national genetic evaluations as an indicator of reproductive efficiency. The objectives of this study were to estimate heat tolerance and evaluate possible genotype-by-environment interaction (GxE) for FSTC in Canadian Holsteins. Phenotypic data comprised 1,964,398 first-parity Canadian Holsteins across 10,313 herds with FSTC records collected between January 1, 2008 and December 31, 2024. Weather data were obtained from the NASA POWER database, including hourly ambient temperature and relative humidity corresponding to the latitude and longitude of each herd (https://power.larc.nasa.gov/; accessed 2026-02-11). Based on previous analysis, daily temperature-humidity index (THI) values were averaged over a window from 3 days prior to 2 days after the first insemination date associated with each FSTC record. A reaction norm model was fitted with an intercept term representing fertility performance under thermoneutral conditions and a slope term representing heat tolerance. A previously estimated threshold of THI 68 was assumed for the onset of heat stress effects, and a heat stress function (HSF) was defined for the slope as HSF = max(0, THI-68). Heritability estimated were 0.02 ± 0.003 for thermoneutral FSTC and 0.05 ± 0.02 for heat tolerance of FSTC. The genetic correlation between fertility and heat tolerance was moderately unfavorable (-0.31 ± 0.26), indicating GxE and a potential trade-off between reproductive performance and resilience to environmental pressures.

Phenotypic analysis of heat stress effects on enteric methane emissions in Holsteins

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In response to climate change targets, reducing enteric methane (CH4) emissions has become a priority for the dairy sector. Genetic selection provides a scalable mitigation strategy, and mid-infrared spectroscopy (MIRS)-predicted CH4 is a practical proxy trait for large-scale genetic improvement. Concurrently, rising global temperatures are intensifying heat stress (HS), with well-documented consequences for dairy performance and profitability. The temperature humidity index (THI) combines ambient temperature and relative humidity to reflect an animal's thermal load. However, the way HS interacts with enteric CH4 emissions remains unclear. Thus, the objective of this study was to identify the THI threshold at which MIRS-predicted CH4 is affected by HS in Canadian Holsteins. The dataset included 743,106 test-day records from 524,832 first parity Holstein cows in mid-lactation, collected between 2018 and 2025. A univariate repeatability model was used to adjust the MIRS-predicted CH4 phenotype by accounting for all known sources of genetic and environmental variation to extract residual CH4. Average hourly THI was calculated from ambient temperature and relative humidity obtained from the NASA POWER database (https://power.larc.nasa.gov/; accessed 2026-02-12) and averaged across 24 hours to obtain daily THI. The accumulated effect of HS was examined over five time windows ranging from the test-day alone to the test day plus the four preceding days (i.e., 5-day average). A segmented polynomial regression was used to identify the breakpoint at which MIRS-predicted CH4 changes with rising THI. Estimated thresholds ranged from THI 67 to 70 across time windows. A 2-day average THI window showed the best model fit (R^2=0.69) and the greatest post-threshold slope (0.57 ± 0.10), making it the most informative window for capturing HS effect on CH4. The results of this study indicated that CH4 emissions increased above the THI threshold of 70, corresponding to HS conditions. These findings suggest that HS effects on MIRS-predicted CH4 occur more sharply within shorter time windows. In future analyses, the identified THI threshold will be used to assess the genotype-by-environment interactions through reaction norm models, characterizing individual MIRS-predicted CH4 performance below and above the threshold. Keywords: Heat stress, MIRS-predicted methane, Temperature humidity index

Genotype by environment interactions for methane emissions of dairy cows fed 3-nitrooxypropanol

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Reducing methane (CH4) emissions of ruminant livestock is important as ruminants are the single largest CH4 emitter in the agricultural sector. Several methods exist to reduce enteric CH4 emissions of cattle and two of the most promising methods applied in practice are the provision of CH4 reducing feed additives and genetic selection for low CH4 emissions. It remains unclear if combining dietary strategies and genetic selection can result in a larger reduction of CH4 emissions, or that the two strategies interact and may negatively influence each other. Therefore, our aim was to investigate if genotype by environment (G x E) interaction is present between animal breeding and dietary strategies that aim to reduce enteric CH4 emissions. Data were available on enteric CH4 emissions of cows recorded by 'sniffers' (WD-WUR v1.0 and v2.0, manufactured by Carltech BV) from the feed bin of milking robots, where several cows were provided 3-nitrooxypropanol (3-NOP; marketed as Bovaer®, dsm-firmenich AG, Kaiseraugst, Switzerland) during the CH4 recording period. Records up to 405 days in milk were included of cows with a minimum breed fraction of 75% Holstein. In total, 55,998 weekly average CH4 measurements were included in the analyses from 5,102 cows on 52 farms, of which 2,446 measurements from 453 cows on 8 farms with 3-NOP supplementation. Of these, 171 cows on 4 farms had CH4 records with and without 3-NOP supplementation. The average CH4 concentration for all 453 cows receiving 3-NOP was 493 ppm, whereas the average CH4 concentration was 620 ppm for cows without 3-NOP supplementation. The genetic correlation between CH4 with and without 3-NOP supplementation was estimated with a bivariate animal model using a restricted maximum likelihood approach. The model included fixed effects for herd × year × week, 2nd order Legendre polynomials for age at calving (in days), parity × 3rd order Legendre polynomials for days in milk, second breed × 2nd order Legendre polynomials for the second breed fraction, and random effects for the genetic cow effect, two permanent environmental effects for the repeated measurements for each cow within and between parities, and the residual effect. The genetic correlation between CH4 recorded with 3-NOP supplementation and CH4 recorded without 3-NOP supplementation was 0.71 ± 0.18. This indicates that there is little G x E interaction, thus selection responses are expected to remain in the desired direction if selection decisions are made using datasets that mix measurements from periods with and without 3-NOP supplementation, albeit s.e. is still large. Further analyses will quantify differences in the CH4 mitigating response to 3-NOP for cows with a high or low genetic merit for CH4 emissions.

Towards a resilience selection index capturing health, longevity and heat tolerance in three German dairy breeds

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Resilience, defined as an individual's ability to cope with short-term external stressors and to recover as fast as possible, has become a central topic in animal breeding research in recent years. In various livestock species, different indicator traits for resilience have been studied. The most promising approaches are based on changes in longitudinally recorded traits, such as the variance and autocorrelation of daily milk yield in dairy cows. While variance reflects fluctuations in milk yield — where lower values can be interpreted as higher resilience — autocorrelation indicates recovery time, with values close to zero being desirable. Previous studies reported low to moderate heritabilities for resilience indicators and generally favorable correlations with, health, and functional traits, raising the question of how resilience can be integrated into breeding programs. Consequently, a resilience selection index combining multiple resilience indicators has been proposed. In this study, we build on the resilience selection index recommended by Keßler et al. (10.3168/jds.2024-25323) and studied how selection for resilience affects existing breeding values for health, longevity, and heat tolerance. The study was conducted within the KlimaFit project and is based on data from 56 dairy farms equipped with automatic milking systems in Baden-Württemberg, Germany, collected between 2022 and 2024. Data from 2,211 German Holstein, 3,445 German Fleckvieh, and 1,229 German Brown Swiss cows were analyzed. For each lactation per cow, a lactation curve was modeled using P-spline interpolation and limited to days 10 to 305. Based on daily milk yield, several resilience indicators were calculated: the variance of absolute daily milk yield (v); the variance (vd) and autocorrelation (rAuto) of deviations between observed and predicted absolute daily milk yield; the variance of daily milk yield relative to the 305-day yield (vr); and the variance of deviations between observed and predicted relative daily milk yields (vrd). Breeding values were estimated separately for each breed and resilience indicator using an animal model including fixed effects (herd-year-season, lactation number, age at first calving, and data completeness) and random additive genetic and permanent environmental effects. Breed-specific resilience selection indices were constructed by combining single resilience indicators with optimized weighting factors. Optimization aimed to maximize the expected selection response in health and fitness traits using an L-BFGS algorithm. Selection responses in health, fitness, and longevity traits resulting from selection on resilience were subsequently evaluated. Across all breeds, the correlated selection responses in health and fitness traits exceeded the correlated response in longevity. German Holstein showed the highest responses (approximately 0.22 for health and 0.20 for longevity), followed by German Brown Swiss (0.15 for fitness and 0.10 for longevity), while German Fleckvieh showed the lowest responses (0.10 for fitness and 0.05 for longevity). Currently we are in progress of combining the resilience selection index with heat tolerance. The latter one will be analysed using a Broken Stick model to quantify milk yield declines during heat waves. These results will be used to refine our selection index towards including heat tolerance. Overall, our results support the implementation of resilience-based selection across dairy breeds, as it positively affects health and longevity traits and might contribute to heat tolerance.

Recursive model approach to define nitrogen use efficiency in Holstein dairy cows

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Improving nitrogen utilization in dairy production is essential for reducing environmental nitrogen losses and enhancing the sustainability of livestock systems. Nitrogen use efficiency (NUE), defined in this study as the proportion of nitrogen intake converted into milk protein, is a key indicator of both environmental and economic performance. On average, less than half of the nitrogen consumed by dairy cows is retained. Therefore, increasing NUE offers substantial potential to reduce nitrogen excretion, lower feed costs, and improve production efficiency. Because direct measurement of NUE is not feasible at scale, indicator traits that describe nitrogen intake, output, and mobilisation could offer a potential approach. Recursive structural equation models provide a suitable framework for defining NUE-related traits because they represent prospective causal relationships among these indicators. The objective of this study was to define and evaluate a genetically derived NUE-related trait suitable for genetic evaluation. Indicator traits included dry matter intake (DMI) as a proxy for nitrogen intake, milk urea nitrogen (MUN) as a measure of nitrogen excretion, and energy-corrected milk (ECM) plus metabolic body weight (MBW) to represent nutrient partitioning into production and maintenance. The dataset comprised 97,698 records from 2,399 first parity Canadian Holstein cows, with 39,822 animals in the pedigree. Four-trait repeated-record models were implemented in ASReml 4.2. Models included fixed effects of herd-year-season and age at first calving, and classes for days in milk. Genetic, permanent environmental and residual variance components were estimated across days in milk (DIM) 5 to 305. Additionally, repeated records models were fitted using test day records within five lactation stages (early to late). The NUE-related trait was defined using a structural equation model in which MUN was modelled as a nitrogen-output trait conditional on nitrogen intake (DMI) and the energy sinks (ECM and MBW). Heritability estimates for NUE-related trait were moderate across time periods, ranging between 0.23±0.05 and 0.34±0.02. Milk urea nitrogen and DMI heritabilities were low to moderate (0.18±0.05–0.24±0.05 and 0.15±0.03–0.20±0.03, respectively). Heritabilities for ECM were moderate (0.21±0.03–0.27±0.03), while heritabilities for MBW were moderately high (0.38±0.04–0.48±0.06). Repeatability estimates for the NUE-related trait ranged between 0.32±0.04 and 0.37±0.03. These estimates indicate the potential for genetic improvement of the proposed NUE-related trait. Due to the recursive model approach, the NUE-related trait was genetically independent of DMI, ECM and MBW. Genetic correlations between the NUE-related trait and MUN across lactation was averaged 0.70 and ranged between 0.68 and 0.93 across individual lactation periods, indicating that nitrogen utilisation varies across lactation stages. A genetically defined NUE-related trait offers a practical tool to reduce nitrogen emissions through genetic selection while maintaining production. If validated in larger and more diverse datasets, our proposed approach could support incorporation of nitrogen-surplus reduction into routine genetic evaluation and breeding objectives, providing a practical lever to improve environmental performance alongside existing selection for feed and methane efficiency.

A New Multivariate Index for Resilience Selection in the Nelore Breed

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Animal resilience is described by three indicators related to the growth curve: logarithm of residual variance (LNVAR), autocorrelation (RAUTO), and skewness (SKEW), which differ in biological interpretation, scale, and variance, making isolated selection and joint interpretation difficult. This study aimed to construct and evaluate a multivariate genomic index for resilience by comparing a simple standardized index and the optimal Hazel index. (Co)variance components and Genomic estimated breeding values (GEBVs) were obtained for 5,294 Nelore cattle using Bayesian inference. Because the three indicators have distinct biological interpretations, a biological reorientation was first applied (T_LNVAR = −LNVAR; T_RAUTO = −|RAUTO|; T_SKEW = −|SKEW|), ensuring that higher values consistently represented greater resilience. Since the indicators also differed in scale and variance, Z-score standardization was applied (mean = 0; SD = 1) to allow direct comparability. Two indices were then constructed: (i) a simple index (I_RES), calculated as the arithmetic mean of the standardized GEBVs, and (ii) the optimal Hazel index (I_H), derived from $b = P^{-1}a$, where P is the phenotypic (co)variance matrix and a is a vector of equal economic weights. The resulting optimal weights were applied to the standardized GEBVs. The indices were evaluated in three analytical blocks. First, I_RES was assessed through correlations with individual indicators, expected genetic gain (ΔG), and Top 10% male ranking. Second, I_H was evaluated through analysis of optimal weights, ΔG, accuracy, Top 10% ranking, and sensitivity tests removing one indicator at a time. Third, both indices were compared through correlation between indices, Top 10% overlap, ΔG differences, and relative contribution of each indicator. Under equal weighting, SKEW showed the highest correlation with I_RES (0.69), followed by LNVAR (0.65) and RAUTO (0.58), indicating greater relative influence of SKEW on the final score. The expected genetic gain (ΔG) reflected this correlation pattern, with stronger responses associated with indicators more closely related to the index, and the Top 10% ranking defined animals superior under equal-weight selection. When applying Hazel's methodology, the (co)variance structure redistributed the relative importance of indicators, with SKEW showing the highest correlation with I_H (0.70), followed by RAUTO (0.65), while LNVAR presented the lowest contribution (0.56). This reweighting was reflected in ΔG, where RAUTO presented the highest isolated genetic gain (0.58) under I_H. The accuracy of I_H reached 0.80, and sensitivity analyses demonstrated structural robustness, maintaining correlations above 0.85 even after removing any single indicator. The Top 10% ranking under I_H identified animals selected according to optimal weighting. Finally, comparison between indices revealed a high overall correlation (r = 0.99), indicating similar ranking hierarchy; however, Top 10% overlap between I_H and selection based on each indicator individually ranged from 26.5% to 42.6%, indicating that single-trait selection would not identify genetically balanced individuals, and differences in ΔG patterns demonstrated that Hazel's weighting modifies the expected selection response by redistributing emphasis among resilience components. Overall, the proposed multivariate index proved robust and reliable for genomic selection, with the optimal Hazel index improving the distribution of genetic emphasis among resilience components and promoting more balanced genetic progress.

Genomic Insights into Genotype × Feed Interactions for Feeding Behaviour, Efficiency and Carcass Traits in Different Pig Breeds

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Due to environmental pollution and the excretion of unused feed nutrients, feed efficiency in pig production is a highly important topic. Current research topics focus especially on nitrogen (N) and phosphorus (P). However, the pigs' feed efficiency is influenced by numerous intrinsic and extrinsic factors. On the one hand, the nutrient composition of the feed can be adjusted, and on the other hand, the animal's genotype also contributes to this variation. The interaction between these different factors can lead to genotype×feed interactions (G×E). The aim of this study was to assess the influence of breed-specific genomic characteristics on pigs' responses to different feeding regimes. Therefore, two feeding trials were conducted to evaluate the effects of a N/P-reduced feeding strategy on feeding behaviour, feed efficiency and carcass composition traits. Two feeding groups were evaluated, where one group received a control (CON), and the other group a N/P-reduced feed (RED). In total, 1,052 pure bred landrace boars (PB) and 796 crossbreed Piétrain×F1 pigs (CB) (393 males and 403 females) were evaluated. Phenotypes were recorded in the performance test stations of the BHZP GmbH for PB and of the Landesanstalt Schwarzenau for the CB. Six feeding behaviour, six efficiency and ten carcass traits were examined for PB, and six efficiency and seven carcass traits for CB. Genomic DNA was isolated from skin or muscle tissue samples collected routinely after slaughter. All pigs in the study were genotyped using the Porcine 70k ggp porcine Illumina© HD array. Quality control (QC) was performed using PLINK, accounting for a call rate > 0.95 and a minor allele frequency < 0.05. SNPs were annotated on the Pig Sscrofa 11.1, and a Genome-Wide Association Study (GWAS) was performed using the R-package rrBLUP. After QC, 55,378 SNPs for PB and 68,816 SNPs for CB remained in the analyses. First, these data were analysed by considering the feeding group as a fixed effect in the model. This was followed by an analysis within each feeding group, where the data were divided by feeding group. To account for multiple testing, p-value adjustments were performed with Bonferroni correction and the false discovery rate control. A total of 265 and 235 significant SNPs across 12 chromosomes were identified for PB and CB, respectively. For feeding behaviour, 15 significant SNPs were identified. For efficiency traits 42 and 33, and for carcass traits 208 and 202, significant SNPs were identified in PB and CB, respectively. Indeed, the majority of these numbers in CB are for the trait drip loss (125 SNPs). For the within-feeding-group analyses of feeding behaviour, PB animals showed 15 SNPs in both, CON and RED, and all of these were identical. Similar observations were made for feed efficiency in PB, where the CON revealed 29 SNPs, as well as for the RED. In CB animals, the CON revealed a much higher number of significant SNPs (51), whereas the RED showed only 5 significant SNPs associated with feed efficiency traits. In the evaluation of carcass traits, 128 and 46 significant SNPs in CON and 23 and 51 significant SNPs in RED were identified for PB and CB animals, respectively. The next step is to investigate the identified regions for candidate genes and functionally relevant variants.

Deep phenotypes of growing Charolais cattle divergent in residual feed intake fed two contrasting diets

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Improving feed efficiency (FE) is a major goal to preserve resources and reduce environmental impacts of animal production. Residual feed intake (RFI) is a promising genetic selection metric due to its low correlation with performance indicators, with lower-RFI animals being more efficient. However, RFI classification is poorly reproducible when a high-starch (HS) diet is replaced by a high-fiber (HF) diet, indicating a genotype × environment (G×E) interaction. Deep phenotypes of FE, representing inner meta-mechanisms, may relate more closely to genotype than RFI but remain unexplored in beef cattle. This study estimated deep phenotypes of low- and high-RFI Charolais bulls fed HS or HF diets and assessed G×E interactions. Deep phenotypes were estimated using the mechanistic, dynamic model LiGAPS-Beef, a genetic algorithm, and measured phenotypes. LiGAPS-Beef simulated bulls with a daily timestep based on animal genotype, climate and available feed quality and quantity. Data from an in vivo trial with low-RFI (n =16) and high-RFI Charolais bulls (n = 16) fed HS or HF diets were used to calibrate the model. Four measured phenotypes (body protein and lipid content at d0, d84, and d200, daily DMI and fortnightly LW recordings), were compared to simulated phenotypes of individual bulls. Using the genetic algorithm (R package GA), differences between simulated and measured phenotypes were minimized by adjusting 7 deep phenotype values via relative root mean squared errors (rRMSE). Traits adjusted were net energy for maintenance (NEm), Gompertz curve slope (DPAR), maximum feed intake capacity (PHFEEDCAP), efficiency of protein and lipid accretion (PROTEFF and LIPIDEFF), maximum carcass fraction (CFRMAX), and maximum total body weight (TBWMAX). ANOVA tests compared these traits between the two RFI groups and diets. Pearson correlations (r) were used to explore their relationships with measured FE, RFI, and 285 muscle metabolites quantified at slaughter by mass spectrometry (MxP Quant 500 Assay, Biocrates). After minimization, rRMSE across the four phenotypes was 43%, corresponding to an average of 10.6% per phenotype, indicating measured and simulated values were relatively close. Average values of deep phenotypes were 258.1 ± 13.3 kJ NE $kg^{-0.75}$ d^{-1} for NEm, 0.84 ± 0.08 for DPAR, 120.2 ± 3.5 g DM $kg^{-0.75}$ d^{-1} for PHFEEDCAP, 0.63 ± 0.14 for PROTEFF, 0.59 ± 0.12 for LIPIDEFF, 0.46 ± 0.02 for CFRMAX and 1303 ± 74 kg for TBWMAX. All the deep phenotypes were positively ($r > 0.5$) or negatively ($r < 0.5$) correlated ($p < 0.05$) with measured FE during the RFI classification test (d0 to d84), except for PROTEFF ($p = 0.4$). No significant correlations were observed between measured RFI and the deep phenotypes. Consistently, deep phenotypes did not differ significantly between the two RFI groups. In contrast, diet had a strong effect on deep phenotypes, as all estimated values differed significantly ($p < 0.001$) between the HS and HF diets. All of the deep phenotypes were significantly correlated with numerous muscle metabolites, notably those of lipid metabolism such as Phosphatidylcholines and Lysophosphatidylcholines ($r > 0.6$ or $r < -0.6$, $p < 0.001$). In conclusion, although diet effects were still strongly reflected in the seven estimated deep phenotypes, these traits showed strong associations with FE and metabolical indicators. This highlights the potential for further exploration of deep phenotypes in breeding programs aimed at improving FE. Future research is required to link individual deep phenotypes to the animals' metabolic pathways.

Environmental and Spatial Drivers of Genetic Variation in the Rubia Galega Cattle

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Understanding the relationship between genetic variation and environmental heterogeneity is essential for interpreting population structure and informing breeding strategies in livestock. This study investigated whether spatial and environmental factors contribute to the genetic structuring of the Rubia Galega (RG) cattle breed, a native beef population from northwestern Spain that has undergone recent reproductive intensification. We hypothesized that, despite the overall weak genetic structure previously reported for the breed, geographic distance and environmental gradients could still explain subtle patterns of genetic differentiation.Genomic data from 5,275 RG cattle genotyped with a medium-density SNP array (46,280 markers after quality control) were analysed. To evaluate isolation by distance, pairwise genetic differentiation among municipalities was calculated using FST estimates, and geographic distances were obtained from municipal coordinates using the Haversine formula. The relationship between both matrices was assessed through Mantel tests with 1,000 permutations and bootstrap confidence intervals. Sensitivity analyses were performed by excluding municipalities located outside Galicia and by restricting the dataset to animals likely derived from natural mating in order to reduce the potential influence of artificial insemination. In addition, redundancy analysis (RDA) implemented in the VEGAN R package was used to explore the association between allele frequencies and environmental variables, including altitude and temperature-related metrics, together with spatial coordinates. Multicollinearity among predictors was assessed using variance inflation factors, and model selection was conducted through a backward permutation-based procedure.Mantel tests revealed a weak but significant positive correlation between genetic and geographic distances ($r = 0.225$; $p < 0.01$), indicating a moderate pattern of isolation by distance. When municipalities outside Galicia were excluded, the correlation decreased but remained significant, suggesting that part of the signal is influenced by the most distant populations. Analyses restricted to animals likely derived from natural mating produced comparable correlation values despite a substantial reduction in sample size, supporting the robustness of the spatial signal. The RDA model identified mean temperature, minimum temperature, and latitude as significant predictors of genetic variation; however, the proportion of explained variance was small (adjusted $R^2 = 0.015$), indicating a limited influence of environmental gradients on population structure.Overall, these results suggest that the genetic structure of RG cattle is extremely weak but not entirely absent. Subtle spatial patterns are detectable and likely reflect a combination of demographic processes, gene flow, and management practices rather than strong local adaptation. The findings highlight the importance of integrating spatial and multivariateapproaches when interpreting genomic data in livestock populations with high connectivity, and provide useful insights for refining breeding and conservation strategies in the breed.

Effect of temperature and humidity index on the performance of hyperprolific sows at first service

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To determine the influence of temperature and humidity index (THI) on the performance of hyperprolific sows at first service, reared in a warm environment, a prospective comparative observational study was conducted in northwestern Mexico. Four hundred ninety-seven first-time sows from the the PIC Camborough® line preselected as future breeders from the moment of birth were used. The sows were classified into three temperature and humidity index (THI) groups, ranging from 84 to 148 d and from 149 to 268 d of age (≤74, >74 <78 and ≥78 <84). ANOVA was applied to the performance data ($p \leq 0.05$). In gilts that were in an THI ≥74 between 84 and 148 d of age, an older age at puberty (235 vs. 215 d; $p = 0.001$) and at service (268 vs. 238 d; $p = 0.001$) was observed compared to those that were in an THI ≤74; similarly, in sows in an THI ≥74 between 149 and 268 d of age, the age at puberty (239 vs. 220 d; $p = 0.008$) and the age at service (268 vs. 247 d; $p = 0.001$) increased. The observed results allow us to conclude that a THI ≥74 delays the age at puberty and at service in gilts. This variable should be considered in a selection and management program for hyperprolific first-time sows, raised in warm environments, with the aim of increasing the percentage of first-time sows served at an age less than 240 d. Keywords: Gilt, growth rate, THI, puberty, age at service.

Estimation of genetic parameters for egg weight across the laying cycle in hens

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Egg weight is a key trait in modern laying hen breeding programs, especially under extended production systems that reach up to 90 or 100 weeks of age. Maintaining a stable egg weight throughout the laying cycle is essential for market value, production efficiency, and egg quality. The objective of this study was to estimate heritability and genetic correlations for egg weight at different stages of the laying cycle in purebred and crossbred commercial populations, using a multi trait approach. Data were obtained from two pure lines and their reciprocal crossbred populations. Egg weight was recorded at 28, 40, 70, and 90 weeks of age. Purebred hens were housed individually under nucleus conditions, while crossbred hens were kept in commercial environments and recorded as cage means. All the hens from one cage share the same sire. Egg weight at each age and in each genetic type was considered a different trait, resulting in eight traits analyzed simultaneously using a sire multi trait model. Variance components were estimated with a Bayesian approach based on Gibbs sampling. Descriptive results showed that egg weight increased with age in all lines. Line B consistently produced heavier eggs than Line A. Crossbred hens produced heavier eggs than purebred hens at all ages, reflecting heterosis and genetic complementarity. Differences between reciprocal crosses suggested the presence of maternal and non additive genetic effects. Between 70 and 90 weeks, egg weight tended to stabilize in the pure lines, which agrees with current breeding goals aimed at avoiding excessive egg size at the end of the cycle. Heritability estimates in purebred lines were high across all ages, ranging from 0.57 to 0.87, indicating strong genetic control of egg weight, even at late stages of production. In crossbred populations, heritability was lower than in purebreds, ranging from 0.42 to 0.71. Across populations, the heritability estimates were relatively high. These elevated values may be partly explained by the structure of the sire model and the group housing of crossbred hens (75% full sisters), where common environmental and non-additive effects could have inflated the sire variance and, consequently, the additive genetic variance. Genetic correlations between adjacent ages were high and decreased as the time interval increased, this pattern may indicate some changes in gene expression over the laying cycle. Genetic correlations between purebred and crossbred performance were moderate to high for most ages, indicating that selection in pure lines can effectively improve egg weight in commercial crossbreds. However, correlations were lower at 90 weeks, possibly due to environmental differences and increased biological stress in extended production. In conclusion, egg weight shows substantial genetic variation throughout the laying cycle, including late production stages. These results support the use of multi trait genetic evaluations and the integration of purebred and crossbred information to improve long term egg production efficiency.

(Epi)genomic regulation of phenotype diversity and plasticity in cattle

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Cattle husbandry in Europe increasingly moves forward to new farming systems due to animal welfare demands like outdoor contact and changes in landscape use (e.g. grassland). However, this development comes with environmental challenges e.g. heat stress or novel or recurring pathogen exposure in addition to or on top of already existing intrinsic factors like e.g. metabolic imbalances in high-yielding dairy cows. This generates needs for adaptation at the population (via selection) and individual level (via epigenetic modulation on top of an individuals' genetic makeup). Understanding the molecular level of genetic and epigenetic regulation will help evaluating (patho)physiological response mechanisms to biotic and abiotic noxes and indicate potential mitigation options. Main current extrinsic and intrinsic challenges in cattle comprise management stress, infectious pathogens and early lactation energy deficit of dairy cows. Tissue-, ontogenetic-stage and breed-specific distribution of regulatory regions within the genome are the precondition for phenotypic variation and tailored response pattern to such challenges. Thus, respective comprehensive inventories of the bovine genome for core physiological tissues were established within many global and international projects. Particularly within the Functional Annotation of Animal Genomes (FAANG)-associated global and European consortia maps of epigenetic marks for open chromatin and methylation sensitive pattern were assembled. Results demonstrated a match between e.g. metabolic tissue activity and methylation level when lactating mammary gland exhibited the lowest and subcutaneous tissue of male adults the highest whole-genome methylation level. Bovine embryos at 7-8 days of age revealed differences at the transcript as well as methylation level either associated with direct mimicking effects of subclinical maternal ketosis or with effects of heat stress on sperm used for in-vitro embryo production. Interestingly, subsequent heat stress models provide tentative indication for long-term transgenerational effects arising during spermatogenesis. Epigenomic marks during ontogenesis or in early life bear the potential for prediction of phenotype to assist selection or induce countermeasures to ameliorate negative environmental effects. Particularly long-term acquired epigenomic marks provide opportunities for objectively monitoring exposure to adverse extrinsic or intrinsic factors and might provide e.g. summarizing readouts for animal wellbeing and animal welfare potential.

Exploring blood methylome differences in dairy cow breeds under feed restriction

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Understanding DNA methylation changes induced by nutritional stress is essential to decipher the epigenetic mechanisms underlying adaptive capacity and resilience to metabolic challenges in dairy cows. Here, we investigated the impact of short-term feed restriction (FR) on whole blood DNA methylation profiles in Holstein (HOLS, n = 18) and Montbéliarde (MONT, n = 18) multiparous cows. As part of the GenTORE project (Pires et al., 2025; PMID:39521419), cows underwent a nutritional challenge consisting of a sequence of four 4-day feed restriction periods, during which feed allowance was restricted to 50% of individual NEL requirements calculated before the challenge. The first FR was initiated at 87 ± 9 DIM. Whole blood samples collected before (d0) and after (d4) this first FR wave were used to profile DNA methylation using the RUMIGEN EpiChip targeting 44,053 CpG sites. Cell-type proportions were estimated using a deconvolution approach based on an in-house reference dataset. CpGs with a standard deviation < 0.01 or > 20% missing values were removed, and B-values were converted to M-values. Differential methylation analysis was conducted using a Breed × Time linear model implemented in limma, including the combined Breed x Time factor and estimated cell-type proportions as covariates. Repeated measures (paired d0–d4 samples) were accounted for by estimating within-animal correlation and fitting cow as a blocking factor. Technical/batch effects were evaluated and included in the model when they varied within animal. The following contrasts were tested: (i) FR effect within MONT (d4 vs d0); (ii) FR effect within HOLS (d4 vs d0); (iii) breed differences at d0; (iv) breed differences at d4; and (v) the breed × FR interaction. P-values were adjusted for multiple testing using the Benjamini–Hochberg procedure (FDR < 5%). Deconvolution analysis indicated that short-term FR significantly altered lymphocyte and neutrophil proportions ($p < 0.05$) in both breeds, reflecting immune responsiveness to nutritional stress and supporting their inclusion as covariates in the differential analysis. For the FR effect within MONT and HOLS breeds, no differential methylation sites (DMCs) were detected, indicating no short-term DNA methylation changes in response to FR in either breed. In contrast, 2,185 and 1,292 DMCs were detected between breeds at d0 and d4, respectively, with 904 DMCs consistently observed across both time points, highlighting breed-specific epigenetic differences. Interestingly, these 904 DMCs target 185 genes, encompassing genes associated with immune system regulation (e.g., ARG2, ZBTB1, F2RL1, ARG2, RNASE6). Overall, these results indicate that short-term FR has limited effect in blood methylome but reveal baseline epigenetic divergence between HOLS and MONT breeds. These breed-specific signatures may contribute to differences in metabolic adaptation and resilience, warranting further investigation under prolonged or repeated nutritional challenges, and in response to the transition from late gestation to early lactation.

Environmental modulation of blood DNA methylation in Jersey cattle with shared genetic background

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Understanding how environmental conditions shape the bovine epigenome is essential for advancing precision breeding and management strategies. We investigated genetic and environmental contributions to DNA methylation in Jersey cattle using a half-sib design. Blood samples were collected from 440 cows, daughters of 23 Danish Jersey bulls, raised in two contrasting production environments: Denmark (DK; n = 223) and South Africa (SA; n = 217). In addition, semen samples from 18 Danish Jersey bulls were included for typing for methylation. Genomic DNA was extracted from blood or ear clip samples and genotyped using the Eurogenomics SNP array. Only autosomal SNPs were considered for downstream analyses. Quality control was performed using PLINK v1.9, where individuals and SNPs with low call rates were excluded (–mind 0.10, –geno 0.10). SNPs with a minor allele frequency (MAF) below 1% were removed. Sporadically missing genotypes were imputed using Beagle v5.5. After quality control, the final genotype dataset consisted of 45,180 SNPs. For population structure analysis, linkage disequilibrium pruning was applied using PLINK (–indep-pairwise 50 5 0.2), resulting in 4,881 SNPs used for principal component analysis (PCA). DNA extracted from whole blood and semen samples was epigenotyped using the RUMIGEN EpiChip. Methylation data were obtained for 44,053 epigenetic markers. Probes showing zero variance across samples were removed, leaving 42,823 probes for further analysis. Missing methylation values were imputed using the per-probe mean and were used for downstream analyses. Differences in methylation levels between DK and SA cows were tested for each probe using a t-test. Genotype-based principal component analysis (PCA) showed no population stratification between DK and SA cows, with daughters clustering by sire, confirming a shared genetic background. In contrast, PCA of blood DNA methylation profiles revealed a moderate separation between DK and SA cows, and clustering by sire was absent. Overall methylation levels were higher in Danish cows, with a greater representation of both hypo- and hypermethylated CpG sites. The top 25 CpGs significantly different in DK and SA cows showed higher average methylation levels in cows from DK compared to cows from SA Africa. Although we observed a moderate country-specific separation in PCA, clustering based on the top 100 differentially methylated CpGs did not result in a clear separation of cows by country. These results demonstrate that bovine blood DNA methylation from highly similar genetic backgrounds is strongly modulated by environmental conditions, highlighting the epigenome as a sensitive interface between genotype and environment. Ongoing work will explore associations between methylation variation and phenotypic traits in Danish Jersey cows. Overall, the study supports the integration of epigenomic information into dairy cattle breeding programs to improve biological understanding of complex traits and to advance precision breeding and management strategies.

Intergenerational and transgenerational match/mismatching effects of ancestor calving months on lactation curves in Simmental cattle

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The impacts of climate change are becoming an increasing concern for the livestock sector, even in temperate areas. Inter and transgenerational studies in dairy cattle shown that the calving season of female ancestors—used as an indicator of the environmental conditions experienced during pregnancy—can influence the performance of their female descendants. In particular, negative effects of heat stress experienced during pregnancy on offspring performances have been observed. A possible explanation of these results is the occurrence of epigenetic inheritance. According to the match/mismatch theory suggested for interpreting epigenetic programming effects, matched ancestor pregnancy/offspring production environmental conditions may result in offspring advantages compared to mismatched ones. Aim of this work was to investigate the effect of matched/mismatched environmental conditions on milk yield of Italian Simmental cattle breed. A total of 1,119,392 Test Day (TD) records of 125,252 lactations of 59,182 Simmental cattle were analyzed. For each cow, data from the great granddam (GGD) were available. Data were analyzed with a linear model that included parity, combination between GGD and cow calving seasons (SEA_combination), and the interaction between days in milk class and SEA_combination as fixed effects and animal and herd-test date combination as random effects, respectively. The SEA_combination factor was included in the model for accounting for match/mismatch conditions. SEA_combinations affected significantly TD milk yield ($P<0.001$). The lowest least squares means of TD records were observed when GGD calved in autumn or winter (i.e., the best condition) and the cow calved in summer (i.e., the worst condition). On the contrary, largest TD means were obtained for GDD calving in summer and autumn (best condition) and cow calving in summer (worst condition). Results of the present study confirm the importance of the combination between calving and production season of the cow together with calving season of the ancestors in affecting production performances.

Impact of late gestation heat stress on progeny's health in dairy cows

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Heat stress (HS) experienced by gestational dairy cows has been shown to impact on their offspring, particularly on both passive and developing immune competence of the calves. We evaluated the impact of a vaccine challenge on the immune response of in utero HS heifers. The objective of the study was to describe the immune response of heifers exposed to acute (experiment 1) and chronic (experiment 2) HS in utero, in relation to their dams' strategies to cope with HS, and to investigate epigenetic signatures associated with these responses (experiment 3). In experiment 1, 24 Holstein dairy cows and their progeny were monitored, and in experiment 2, 12 dams/heifer couples were monitored. In experiment 3, all dams/heifer couples were monitored to investigate the impact of HS challenge on the epigenomic profile of blood-derived immune cells. To classify the dams according to their adaptation strategies to cope with HS, a Hierarchical Classification on Principal Components (HCPC) was performed on dams centered indicators : ruminal temperature, activities (ruminating, eating, resting and panting behavior), milk production, milk fat content, milk protein content, serum cortisol, serum non esterified fatty acids, serum thyroxin, calves 'weight and serum immunoglobulin of calves. In experiment 1, algorithm discriminated five clusters based on cows' adaptation to HS and in experiment 2, two clusters were discriminated. To classify the heifers according to their response to the immune challenge, a HCPC was performed on cytokines IL1-β, IL-6, IL-8 and IL-10 longitudinal response, specific antibodies for BPI3, BRSV, and Mannheimia haemolytica before vaccination and after vaccination, and specific antibodies for AdenoV and Mycoplasma bovis along with health-related variables and growth variables . In experiment 1, the HCPC revealed four subgroups with distinct cytokines and antibodies patterns and growth outcomes. In experiment 2, the HCPC revealed 2 different subgroups. In experiment 3, DNA methylation was profiled using the RUMIGEN EpiChip to estimate the proportions of three major immune cell types (lymphocytes, monocytes and neutrophils) before and after the vaccination challenge, and to identify potential epigenetic biomarkers. A contingency table between dams' clusters and heifers' clusters did not show any link between the two. Neither did the description of maternal clusters with the heifer's specific variables. However, a significant (pvalue<5%) difference in the proportion of lymphocytes was detected before and after vaccination in heifers born from the more affected cows, whereas this pattern was not observed in heifers born from the less affected cows. Although limited, differential methylation analysis comparing the methylome profiles of heifers born from more affected cows before and after the vaccination challenge revealed four CpGs co-localized with a candidate gene related to immune system function. Our work has highlighted that animal responses to the vaccine challenge varied, but these do not seem to depend on how their dams coped with HS during late gestation. Nevertheless, epigenomic analyses suggest that prenatal HS exposure may modulate immune cell dynamics during later-life immune challenges. Immune responses depend on several factors, both innate and environmental, among which the intergenerational impact of HS appears to have little influence in our region.This project has received funding from the European Union's Horizon 2020 programme for research and innovation under grant agreement no. 101000226.

Epigenome-wide association of DNA methylation with phenotypic traits and environment in French Holstein cows

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To date, large-scale epigenome-wide association studies (EWAS) in livestock remain scarce due to the lack of high-throughput method to obtain DNA methylation data. In this work, we harnessed the RUMIGEN EpiChip, a cattle-specific DNA methylation array, to investigate whether variation in blood DNA methylation is associated with agronomic performance and reflects exposure to different conditions in dairy cows. An EWAS was conducted in 4,608 French Holstein cows from 261 herds. Blood DNA methylation was quantified at 44,053 CpG sites using the RUMIGEN EpiChip; 38,859 probes were retained after quality control. Thirty-three traits were analysed as yield deviations from the national genetic evaluation system, including milk production and composition, fertility, udder health and morphology. Environmental variables were available for 2,945 cows and comprised grazing duration, ration type, dry-off management and herd target age at first calving. Associations between CpG methylation (M-values) and traits were tested using linear models including technical effects, estimated leukocyte proportions, age and the first five genomic principal components to account for population structure. Multiple testing was controlled using Bonferroni correction (adjusted pvalue < 0.05). Significant associations were predominantly detected for milk traits: 72, 61, 52, 35 and 34 CpGs were associated with fat content, protein yield, milk yield, fat yield and protein content, respectively. Fertility traits showed fewer signals (32 CpGs for heifer conception rate and ≤ 2 for other traits), while most morphological traits displayed limited associations. In contrast, environmental factors were associated with a substantially larger number of CpGs: grazing duration (1,121 CpGs), ration type (690), target age at first calving (465) and dry-off management (61). Associated CpGs were distributed across multiple chromosomes, suggesting widespread epigenomic responsiveness to management practices. Overall, our results demonstrate that blood DNA methylation is linked to both production performance and farming conditions in dairy cattle. The stronger signals observed for environmental variables support the sensitivity of the bovine methylome to management-related exposures. Although causality cannot be inferred, DNA methylation may represent an intermediate regulatory layer connecting genotype, environment and phenotype.

Seasonal investigation of circulating microRNAs in two Sicilian cattle breeds to evaluate the response to heat stress

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Global warming and the more complex phenomenon of climate change represent the most relevant and topical emergencies worldwide. Projections suggest that Europe will warm significantly in the coming decades, especially in Mediterranean regions, which are already experiencing the impacts of global warming. Livestock systems are often considered among the main drivers of global warming and, at the same time, suffer the negative effects of climate change. In particular, hot climate and heatwaves strongly compromise the productive and reproductive capacities of dairy cows. In this context, native breeds raised in Mediterranean regions often show greater adaptability to warm climates than breeds bred in temperate environments. The response to heat stress is a complex biological process involving the activation of molecular pathways and the regulation of gene expression at the transcriptional and post-transcriptional levels. MicroRNAs (miRNAs), small endogenous non-coding RNAs, are important post-transcriptional regulators and are involved in numerous biological processes, including response to environmental stress. The objective of this study was to analyze seasonal variations in circulating miRNA profiles of two native Sicilian cattle breeds, Modicana and Cinisara, reared in extensive system, and to identify breed-specific molecular responses. A total of 36 serum samples from the two Sicilian cattle breeds, sampled during the winter and summer of 2025, were analyzed. The QIAseq miRNA library kit was used for library preparation, followed by sequencing on the Illumina NovaSeq X Plus platform in 150-bp single-end mode. Differential expression analysis was performed with DESeq2, in which miRNAs with a false discovery rate (FDR) lower than 0.05 and |log2 fold change| ≥ 1 were considered significantly differentially expressed. A total of 36 miRNAs were significantly differentially expressed between the seasons in the Modicana breed, and 9 miRNAs were detected in Cinisara. In Modicana, several miRNAs identified (including miR-29 family members, miR-145, and miR-2285) have previously been linked to heat stress responses in bovine mammary tissue and blood circulating fractions. Moreover, miR-30-P1d_5p, which was upregulated in summer in this breed, belongs to the miR-30 family, previously associated with heat stress responses in cattle serum and fibroblasts, as well as in oxidative stress and immune-related pathways. Finally, worth noting is miR-423, which is suggested to regulate chronic heat stress responses in Holstein cows, and was found to be downregulated in the summer season in Modicana. In Cinisara, miR-155, a key regulator of immune and inflammatory processes, shows downregulation in summer. Three miRNAs (miR-335-5p, miR-193-P1a-5p and miR-3432-5p) are found in both breeds, but miR-335-5p and miR-193-P1a-5p are regulated differently in each season. The distinct circulating miRNA signatures observed between Modicana and Cinisara suggest different molecular strategies for maintaining physiological homeostasis under summer heat conditions, which could provide new insights into breed-specific adaptation to climate stress.
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Seasonal effects on bull sperm-borne sncRNA profile

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Investigation of seasonal variations in the small non-coding RNA (sncRNA) profile of Holstein bull sperm offers a window into their functionality, especially considering that ejaculated sperm are transcriptionally silent. As non-seasonal breeders, this may hold particular economic importance to improve breeding strategies in the dairy industry. The objective of this study was to characterize seasonal expression profiles of bull sperm-borne sncRNAs, establishing a foundational overview of their temporal dynamics. Semen samples from 21 Holstein bulls from SYNETICS (France) were collected in July, September and December and ranging between 11-24 months old in July. Total RNA was then extracted from all 63 sperm samples and subjected to UMI deep sequencing on Illumina HiSeq2000 (Qiagen, 20 M single-reads (75 bp) per sample). After quality control, reads were processed and annotated using PAQmiR, an in-house pipeline based on miRDeep2 that also enables tsRNA, rsRNA and piRNA annotation. Differential expression analysis was performed using R(DESeq2), which involves performing a likelihood ratio test to identify sequences that showed a significant (Benjamini-Hochberg, adjusted p-value <0.05) change in expression across all 3 seasonal periods. Next, hierarchical clustering was conducted to group significant sncRNAs with shared expression patterns. Gene targets for the miRNAs in each cluster were retrieved using TargetScan v8.0 to identify the significantly enriched (Benjamini-Hochberg, False Discovery Rate <0.05) KEGG pathways and biological processes. After sequencing and annotation, expressed sncRNAs comprised 36.4% of miRNAs, 30.1% of tsRNAs, 10% rsRNAs, 12.9% piRNAs and 10.6% of other sequences. Differential expression analysis revealed 2581 sequences clustered into 4 groups based on their shared expression trends. Interestingly, dominant sncRNA expression varied by cluster: miRNA (34.4%, Cluster 1), piRNA (80.1% – majority of which have a 5' 1U bias, Cluster 2), other sequences (38.6%, Cluster 3) followed closely by tsRNA (31.1%, Cluster 3), and rsRNA (36.4%, Cluster 4). miRNA targets were analysed to identify functionally enriched pathways and processes. Functional enrichment analyses show that the calcium signalling pathway and metal ion transport processes that are known to play an important role in sperm motility and capacitation were statistically enriched. By providing a comprehensive seasonal catalogue of sncRNA subtypes, this study contributes to enhance knowledge on molecular response to seasonal shifts.

Changes over time, individual variability and environmental influence on blood cell counts in healthy heifers

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Calf immunity is of primary importance in cattle production because of the influence of calf health on the subsequent performances and welfare. In order to better understand this complex process and its long-term effects, a vast longitudinal experiment has started at the INRAE Le Pin research facility, aiming to monitor over 800 female calves and relate biological parameters to health, environmental, genetic, and performance data. As a first step, we investigated temporal changes, individual variability, and environmental influences on blood cell counts in the first healthy heifers. Considered animals were purebred Holstein (n=72) and Normande (n=20) females, born between February 2024 and May 2025, sampled at around 3, 14, 70 and 150 days of age and with no recorded health events. Blood cell counts was determined by using an automated hematology analyzer at the LABÉO veterinary laboratory. Levels of the different blood cell parameters were analyzed using linear mixed-effects models to determine the influence of calf's breed, year of birth, birth ease (human assistance or not), birth weight, management of the dam (heifer, indoor intensive system, grazing system) and difference between the real and the theoretical sampling days. Additionally, for a subset of blood samples, genomic DNA was extracted and used for epigenetic profiling with the RUMIGEN EpiChip. A deconvolution approach was conducted from the DNA methylation values to infer the proportion of neutrophiles, lymphocytes and monocytes present in each sample. These estimated cell counts were compared with those measured by LABÉO. First results show that leukocyte counts increased over time ($P < 0.001$), mainly driven by a sustained rise in lymphocyte populations ($P < 0.001$), while neutrophil, monocyte, and eosinophil proportions declined ($P < 0.001$). Basophil counts increased progressively ($P < 0.001$). Red blood cell indices were also strongly affected by age at sampling ($P < 0.001$), reflecting progressive erythrocyte remodeling. Birth weight significantly influenced several hematological parameters, with lighter calves displaying distinct erythrocyte and leukocyte profiles. Most significant covariate effects were observed at 3 days of age, particularly for red blood cell indices, whereas fewer associations persisted later. Breed effects were transient, mainly detectable at 3 days and reappearing selectively at 70 days, while year-of-birth effects were limited to early basophil-related differences. A large remaining variability existed that was not explained by the environmental effects, suggesting that, aligning with the literature reports, there could be a genetic influence on blood cell counts. Next step will be to integrate more immune parameters to better characterize the trajectory of healthy calves.

Diversity of Toll signalling pathway adaptors as a source for breeding for health traits in Czech Red Pied cattle

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The diversity in the innate immune genes represents a source of variants that can be used for targeted breeding for health and general resilience. Consequently, the variation in the genes encoding the members of the Toll signalling pathway has been screened in the population of Czech Red Pied cattle. Although the breed is a part of the group of Simmental breeds, the differences due to the starting cattle population before the crossing in the 19th century are still indicated by mtDNA diversity. A range of adaptors that interact with the main members of the pathway represent an interest due to the presence of highly conserved regions Toll/IL-1R/R (TIR) and death domains (DD). These regions serve for the formation of complexes like myddosomes and enable the transfer of the signal downstream. Most of new mutations in these regions strongly affect the immune response and are not fixed in the population. The MYD88 gene (myeloid differentiation primary response gene 88) for the adaptor MyD88 was used as a representative of this group of adaptors. The pooled genomic DNA samples from 150 bulls of the CRP production population were sequenced using Illumina HiSeq technology. These data were complemented in the course of hybrid sequencing with the results of PacBio technology. Due to a high GC content (up to 90% locally, 69% in the proximal 500 nucleotides), and high autocomplementarity, an optimised PCR system had to be developed to obtain pooled amplicons for genotyping. Diversity found in MYD88 in frame of the CRP population was extremely low. Only 12 SNPs and 1 insertion with frequency above 0.05 were found throughout the entire gene sequence of 4457 bp on chromosome 22, where only 10 SNPs were located in mRNA.. Only one polymorphism was found in the coding sequence, albeit with a low frequency 0.036. Nevertheless, the nonsynonymous change 11648941C>T leads in this case to a substitution Ser>Phe in the product. Functional consequences can be expected due to the localization of this polymorphism in the centre of the TIR region. It is assumed that the triple interaction with TIR regions of TLR and Mal in myddosomes should prevent the persistence of the new mutation in a population. On the other hand, the presence of a polymorphism 2853C>G changing glutamine for histidine was found in the interacting TIR region of TLR2 in this population and functional complementation is not excluded. Notably, a substantially higher diversity of MYD88 was found in the conserved nucleus herd of the historical CRP cattle, namely 58 SNPs and 1 insertion causing 11 amino acid substitutions. Also a conserved population of the indigenous Czech Red cattle harboured 128 SNPs and 1 insertion leading to 44 aa substitutions. An association study based on the targeted genotyping of the 10 intragenic SNPs and additional SNPs in the adjacent regions is carried out. The main traits checked for correlation are the traits used for the calculation of breeding value index, including early reproductive disorders and calving ease, which are known to be affected by the Toll pathway. The ongoing reconstruction of haplotypes in the MYD88 gene will improve resolution of the association study. The number of published studies on bovine MYD88 is very limited to date, in contrast with the numerous described effects in model species. It is expected that the role of MYD88 polymorphism in animal health will be described in a comparative degree.

Dynamics of sheep sperm sncRNAs during maturation, from testis to ejaculated sperm

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Ejaculated sperm contain a diverse repertoire of small non-coding RNAs (sncRNAs). These constitute a heterogeneous group of subfamilies, such as miRNAs, piRNAs, tRNA and rRNA fragments (tRFs and rRFs respectively). Their roles in fertility are not yet fully understood, although they are essential for the proper functioning of spermatogenesis. Surprisingly, spermatogenesis sncRNAs aren't fully discarded at the end of the processes and a part of the ejaculated sperm content is acquire during the epididymis journey and in interaction with seminal plasma. Several studies suggest the involvement of these sncRNAs in embryonic development via their transfer to the oocyte during fertilization, but their biological functions in this context remain to be elucidated. To study their potential roles in early embryonic development, it is therefore important to thoroughly characterize their expression dynamics along the male reproductive tract. To date, no comprehensive catalog of sperm sncRNAs based on localization has been established in sheep. This study aims to fill this gap by describing the dynamics of sheep sperm sncRNAs. Ejaculated sperm were collected from four fertile rams, as well as from the testis and the epididymis (caput, corpus and cauda). The extracted RNA was sent to Qiagen for library construction and 75-bp single-end UMI sequencing with a target depth of 20 million reads per sample. The sequences were then quantified and annotated using the PAQmiR pipeline. The results showed that the sncRNA profile undergoes significant changes from testis to ejaculated sperm: while piRNAs dominate the testis (49.0% of total expression), ejaculated sperm displays a high a miRNA-driven expression landscape (48.4%), despite only 4% of the sequence diversity. Of these, 81.3% were identified as known miRNAs primarily originating from Ovis aries (52.7%), followed by Mus musculus (14.6%) and Homo sapiens (11.64%), while 18.6% were classified as putative novel miRNAs, all of which originated from 1216 canonical miRNAs. A differential expression analysis (DESeq2) based on the source of acquisition, as well as functional analyses and a comparison with the bovine species are currently underway.

Age, genetics, laying intensity, and housing conditions affect DNA methylation of the oxytocin receptor in female chicken

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Animal welfare monitoring is rapidly becoming a critical concern, with an ongoing search for biomarkers. DNA methylation has the potential to serve as such a biomarker. Therefore, we tested the hypothesis, whether hens of genetically distinct layer lines display divergent methylation patterns at selected behavioral genes in response to housing conditions. L68 and BLA (brown layer lines, low and high laying intensity respectively) and G11 and WLA (white layer lines, low and high laying intensity respectively) were used in a cross over-design. Blood samples were collected from 2 day old chicks and at 454 ± 31 days of age (for each condition n=6 samples were collected; all female). Adult animals were raised in groups until the 16th week of age after which they were either housed in individual cages (one animal per compartment) or small groups of 10 animals with access to group nests, perches, litter, pecking blocks, and an additional sand tank for dustbathing. DNA methylation at the OXT, OXTR, POMC, ESR1, HTR1A, HTR2A, and HTR3A gene were determined using direct bisulfite-PCR sequencing. Methylation levels at individual CpG sites between young and old animals for each gene were compared using t-tests. A three-way ANOVA followed by Tukey's post hoc test was used to determine an interaction between genetics, housing condition, and laying intensity. A two-way ANOVA analysis followed by Tukey's post hoc test was used to test for interaction between genetics and laying intensity for each housing condition separately. False discovery rate (FDR) correction was implemented for each analysis. OXTR was the only gene displaying consistent differences in DNA methylation depending on age, genetics, laying intensity, and housing. The largest effect of age on methylation was evident for OXTR, with adult animals displaying significantly higher methylation than 2-day old chickens. While methylation levels for OXTR did not differ between chicks, significant differences in methylation levels were detected for OXTR when comparing samples from adult animals. Three-way ANOVA revealed a significant interaction effect of laying intensity x housing and a significant interaction effect of genetics x housing on the majority of CpG sites. For some CpG sites, a significant interaction effect of all three factors was present. A follow up two-way ANOVA examined the effects of genetics and laying intensity for each housing condition. For samples obtained from animals housed in a layer cage, except for one CpG site, no effect of laying intensity on mean methylation levels was detected, while genetics had a significant impact on the majority of CpG sites. For samples obtained from animals subjected to group housing, laying intensity and genetics had a significant effect on the majority of CpG sites. When comparing differences between group housed animals versus animals housed in an individual cage, no differences were detected for BLA (brown, high producing), while for L68 (brown, low producing) the majority of CpG sites displayed significantly higher levels of methylation for animals housed in groups, while WLA (white, high producing) presented consistently higher methylation levels in samples obtained from animals housed in an individual cage. Our findings provide evidence that genetics, in interaction with the environment, significantly influences DNA methylation of the OXTR gene. Several studies in humans have shown that OXTR methylation is sensitive to environmental cues. In particular, early-life adversity can increase methylation at specific regulatory CpG sites. Future studies will focus on validating OXTR as a biomarker for animal welfare monitoring.

The effect of ambient temperature during first 10 days after parturition of female calves on their milk production during the first lactation

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Temperature conditions are important for calves' development, health, and welfare especially during the late prenatal and early postnatal periods, under both low and high ambient temperatures. The aim of this study was to evaluate the effect of ambient temperature conditions during the first 10 days after parturition of female dairy calves on their milk production during first lactation. Selected dairy farm focuses on the intensive breeding of Holstein cows. After calving, the female calves were moved within the farm to individual hutches with small fens and fed whole milk. The daily outdoor temperatures during first 10 days after parturition were obtained from a nearby meteorological station. Based on the average temperature, the following temperature groups were created: A: bellow 0 $_{o}$C (N=82), B: 0-15 $_{o}$C (N=422), C: 15-22 $_{o}$C (N=168); and D: over 22 $_{o}$C (N=119). From farm databases, there were available data of 791 dairy cows born within year 2015-2018 for evaluation on their first lactation performance, including milk yield (kg) and fat, protein, and lactose production (kg and %) per 305 days of lactation. The effect of temperature group on milk yield and milk composition was analysed using linear models (PROC MIXED, SAS) with Tukey-adjusted least squares means; differences were considered significant at $P \leq 0.05$. Temperature group had a significant effect on milk yield ($P = 0.0026$). Milk yield differed significantly between Group B (8843±2173 kg) and Groups A (8380±1976 kg) and D (8508±2383 kg). A significant effect of temperature group was also observed on all milk component yields in kg ($P = 0.0024$, $P = 0.0015$, and $P = 0.0012$ for fat, protein, and lactose yield, resp.). For all these parameters, values differed between Groups B and D. Additionally, differences between Groups A and B were observed for protein and fat yields in kg. No significant effects ($P > 0.05$) of temperature group were found for milk composition in %. In conclusion, ambient temperatures below 0$_{o}$C and over 22$_{o}$C during first 10 days of life of female calves' can negatively influence milk production in their first lactation. Supported by "Agroecological Transition of the European Dairy Farming System" (TEDY; CUP I53C24002990006) and by grant LIFE23-IPC-SK-NatAdaptSK.

Prenatal thermal environment of calves and their milk production and composition in first lactation

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Maternal heat stress can have lifelong negative effects on offspring, including impaired immune function and reduced productive performance. The aim of this study was to evaluate the effect of the average ambient temperature during the last 30 days before calving on milk yield and milk composition during the first lactation. The study was conducted using data on milk yield (kg) and milk composition (fat, protein, total fat and protein, and lactose; expressed in kg and %, resp.) from 791 cows on a commercial Holstein dairy farm located in western part of Slovakia. The animals were divided into four temperature groups according to the average ambient temperature during the last 30 days before calving: Group 1: t < 0 °C (N = 63); Group 2: t = 0 – 14.99 °C (N = 402); Group 3: t = 15 – 21.99 °C (N = 215); and Group 4: t ≥ 22 °C (N = 111). The effects of temperature group on milk yield and composition were analysed using linear models (PROC MIXED, SAS) with Tukey-adjusted least squares means; differences were considered significant at P ≤ 0.05. Regarding milk composition (%), a statistically significant effect of temperature group was found for fat (P = 0.0146). Significant differences in fat percentage were detected only between Groups 1 and 2 and between Groups 1 and 3. For milk yield, a significant difference was observed between Groups 2 (8,791.13 ± 2,181.01 kg) and 4 (8,417.01 ± 2,384.90 kg). Regarding milk composition (kg), a significant effect of temperature group was observed for fat (P = 0.0440) and lactose (P = 0.0202). The lowest fat (376.31 ± 62.85 kg) and lactose yield (416.00 ± 72.46 kg), were recorded in Group 4, whereas the highest fat was observed in Group 1 (402.95 ± 68.85 kg), and the highest lactose (437.33 ± 62.35 kg) in Group 2. Maternal exposure to environmental heat stress during the last month of prenatal development may lead to lower milk yield and reduced fat, protein, and lactose production (kg) during the first lactation. Supported by “Agroecological Transition of the European Dairy Farming System” (TEDY; CUP I53C24002990006) and by the grant “LIFE24-IPC-SK-NatAdaptSK-101202890-2“.

Genetic correlations between clinical mastitis, metritis and ovarian cysts in Polish HF cows

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Genetic correlations between clinical mastitis (MA), metritis (ME) and ovarian cysts (OC) in the population of Polish Holstein-Friesian (PHF) cows were estimated. The dataset for health traits was created on the basis of PLOWET database developed by the National Research Institute of Animal Production and included phenotypic data collected in 4 experimental dairy farms. Phenotypic evaluation of health traits of the cows was carried out between 2016 and 2025 and was based on veterinary registration. The dataset used in our study comprised 12,492 records of cows. Gibbs sampling algorithm implemented in the BLUPF90 package was used for the computations. The linear observation models included additive random effects of animal, fixed effects of herd-year-season of calving subclass (HYS) and lactation stage, fixed regressions on cow age at calving and fixed regressions on cow age at the time the disease was diagnosed. Cow and bull pedigrees contained two generations of ancestors i.e. parents and grandparents. Genetic correlation coefficients were ranged from 0.48±0.019 (MA vs OC) and 0.55 ±0.026 (MA vs ME) to 0.73 ±0.012 (ME vs OC). The magnitude of the obtained coefficients of genetic correlation are in the range of coefficients published in the papers of other authors and indicate the possibility of including these results in the national cattle improvement program e.g. to optimize selection indexes.

Transcriptomic analysis to identify genes involved in physiological and immune responses to heat stress challenges in Romanian Holstein lactating cattle

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Heat stress (HS) was shown to negatively impact production, reproduction and health of all livestock species, while having the largest impact on lactating dairy cattle, which are particularly susceptible as the high yields production generate increased metabolic heat loads. The aim of the current study was to investigate genes and molecular mechanisms involved in physiological responses to heat stress challenges in lactating Romanian Holstein dairy cows, throughout a transcriptomic analysis. For this reason, RNA sequencing (RNA-Seq) was performed on whole-blood samples collected from seven multiparous Romanian Holstein cows, under HS (average temperature-humidity index (THI) = 77.5) and thermoneutral (TN, average THI = 31) conditions. Across all analysed samples, RNA-Seq generated a total of 1,070,066,390 raw reads, with an average of approximately 76.4 million reads per sample. Significantly differentially expressed genes (DEGs) were annotated against public databases. Functional enrichment analysis was conducted using clusterProfiler (v4.14.6), identifying enriched Gene Ontology (GO) terms and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways associated with the thermal stress response. Significance tests were performed with the functions enrichGO and enrichKEGG, using default thresholds (0.05 for p-values and 0.2 for q-values). Gene annotations were obtained from the R package. A number of 487 DEGs were identified, of which 447 genes were downregulated and 40 genes were upregulated, during the HS exposure compared to TN episode. The consistency for the down-regulated genes associated with immune function (e.g. CD200, PAX5, BACH2, ANKRD42), metabolic health (e.g. SMAD3, PPARGC1B, NOX1, CCDC30, LCLAT1), and reproductive performance (e.g. AMHR2, ADAM20, CCDC170) underlined the interconnectedness of these biological functions. The simultaneous down-regulation of transcription factors (e.g. PAX5, BACH2) involved in central biological processes such as immune cell differentiation and function, suggests a coordinated weakening of the adaptive immune system, potentially increasing susceptibility to various infectious diseases during HS challenges. The contrasting patterns of gene expression, characterized by a predominant downregulation of genes important for reproduction-related functions and complex adaptive immune processes, alongside the upregulation of immediate protective and antiviral responses, suggests a biological prioritization of survival over reproduction and productivity. These findings provide detailed molecular insight into the physiological challenges faced by heat-stressed lactating Romanian Holstein dairy cows.

Hormonal induction enhances functional differentiation in bovine mammary organoids

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Bovine mammary organoids are an innovative in vitro 3D model for studying lactation biology. To accurately model the mammary gland, organoids must reproduce its cellular architecture and hormonal responsiveness. Organoids should also support the synthesis and secretion of milk components. We hypothesised that lactogenic treatment promotes functional differentiation in bovine mammary organoids leading to enhanced structural organization and upregulation of genes involved in differentiation and lactation. This study aims to investigate the lactogenic differentiation in bovine mammary organoids by comparing the effects of proliferative and differentiation culture conditions using confocal microscopy, milk-related gene expression analysis and secretome profiling. Bovine mammary epithelial organoids were generated from mammary epithelial fragments isolated from the mammary gland of non-pregnant heifers. The organoids were cultured for 20 days in MammoCult™ commercial medium, or for 10 days in MammoCult™ followed by 10 days of lactogenic differentiation. This protocol included a 4-day hormone pre-treatment with estradiol and progesterone, followed by 6 days of bovine pituitary extract (BPE) treatment. All media were supplemented with hydrocortisone at a higher concentration during lactogenic induction. Organoids were stained for confocal microscopy, and gene expression was analyzed by qPCR. Imaging using confocal microscopy showed, in both conditions, that the apical localization of the tight junction protein ZO-1 was oriented towards the lumen. The organization of the ZO-1 network was more well-defined in the organoids cultured with lactogenic treatment than in those cultured in MammoCult™. Preliminary data on gene expression analysis showed that lactogenic treatment increased progesterone and oestrogen receptor expression (PGR and ESR1, respectively), reflecting responsiveness to the hormonal treatment. The lactogenic treatment also modulated the expression of milk protein genes, including β-casein (CSN2) and κ-casein (CSN3), and led to increased lactoferrin (LTF) gene expression which is consistent with the induction of lactogenic differentiation. Taken together, the preliminary findings suggest that lactogenic treatment enhances structural organization of bovine mammary organoids and promotes the modulation of the genes involved in the lactation. Further analysis of gene regulation and expressed proteins secreted into the lumen of organoids will be performed together with comprehensive mass spectrometry-based proteomic profiling to identify milk-associated proteins. These approaches will provide a more detailed characterization, helping to establish bovine mammary organoids as a physiologically relevant model for lactation biology. This organoids model represents a promising tool for understanding mammary function and the production of cell-based milk components.

Genome-wide DNA methylation profiling in a German Black Pied Cattle trio

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DNA methylation regulates gene expression, imprinting, and genome stability. Although reprogramming occurs during gametogenesis, some regions retain parental signatures. This study characterized methylation variations in a German Black Pied Cattle (DSN) trio and its overlap with known imprinted genes. Blood DNA (no cell count available) from a bull (8y), a dam (4y), and their male offspring (6w) was sequenced using PacBio HiFi long-read sequencing (Revio) for direct detection of 5-methylcytosine (5mC) without additional chemical treatment. CpG methylation was quantified at single-base resolution, and methylation levels calculated as the proportion of methylated versus total cytosine reads. Long reads (15–20 kb; Q30 >99.9%) were aligned to the bovine reference genome. Methylated cytosines were assigned to Differentially Methylated Regions (DMRs) using a Bayesian hierarchical model (DSS; Feng et al. 2014), which normalized the data for spatial correlation between sites, read depth, and biological variability. DMRs were annotated relative to promoters (±2 kb), CpG islands and shores, UTRs, exons, introns, and repeats. Genes overlapping DMRs were used for Gene Ontology (GO) enrichment analysis. The average sequencing depth across all animals was 31.92 ±2.75. After normalization, approximately twice as many DMRs were observed between sire–calf (138,034) as between dam–calf (60,214). Mean DMR length and CpGs number were 216 ±245 bp with 9.7 ±9.5 CpGs (sire–calf), and 189 ±188 bp with 10.6 ±13.1 CpGs (dam–calf). The calf generally showed lower methylation levels than both parents across regulatory elements. Promoter methylation was similar to the dam, while 5′UTRs were more methylated in the dam. Compared to the sire, the calf showed reduced methylation levels across promoters, UTRs, gene bodies, and repetitive regions. GO enrichment analysis of genes differentially methylated between sire–calf or dam–calf pairs revealed, in both comparisons, molecular functions significantly associated with binding and protein binding ($p<0.002$). Genes showing similar methylation in one parent–offspring pairing but not the other were significantly enriched ($p<0.05$) for cell morphogenesis, neuron projection guidance, negative regulation of Ras and small GTPase signaling, GTPase activator/regulator activity, synapse and junction components (dam–calf pattern); and regulatory and intracellular signaling processes, phosphatidylinositol-mediated pathways, synaptic and membrane components, cytoskeletal organization, and binding, ion-binding, and GTPase regulatory activities (sire–calf pattern). Assuming that methylation patterns unique to the dam–calf pair indicate maternal imprinting and those unique to the sire–calf pair indicate paternal imprinting, classical paternal imprints such as ATP2C2, EGFL7, THRB remained consistent in the DSN trio. Despite the expected 50% methylation of imprinted regions in somatic cells, genes maternally imprinted in humans or mice, including ANO1, CELF4, CHST8, CTNNA3, FBRSL1, FKBP6, OSBPL5, and SMOC2, appeared paternally imprinted in DSN, while CDH18 and MEAK7 showed the opposite pattern. Genes with complex imprinting conservation, such as GNAS and GRB10, were maternally imprinted in sheep, cattle, or rats but paternally in DSN. Several DMRs overlapped with QTLs for production and conformation traits in DSN, including ABCC5 and FGF12 (milk and protein yield; BTA1), SLC15A5 (fat content), CLEC4A, CD163 (birth weight; BTA5), and RORA (body weight; BTA10). While restricted by a single trio and the lack of blood cell count data, this study demonstrates the utility of long-read sequencing for bovine methylation analysis, though results require cautious interpretation.

Navigating the Promise and Pitfalls of Phytochemically Diverse Pastures for Multifunctional and Sustainable Livestock Systems

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Pastures are increasingly recognized as multifunctional agroecosystems, extending beyond their traditional role as sources of animal feed. Confronted by environmental degradation, input cost volatility, and societal demands for sustainable food systems, pasture-based livestock production must concurrently support productivity, ecosystem services, and environmental stewardship. This expanded mandate necessitates re-evaluating pasture design not only in terms of yield and nutritive value, but also in terms of biochemical functionality, ecological resilience, and animal health outcomes. Phytochemically diverse pastures that incorporate legumes and bioactive-rich forb species offer a promising pathway toward such multifunctionality. Plant secondary compounds, including phenolics, tannins, and terpenoids, have been associated with improved rumen function, enhanced immune responses, reduced enteric methane emissions, mitigation of nitrate leaching, and increased antioxidant capacity of animal-derived products. However, despite growing interest, significant knowledge gaps remain regarding the concentration-dependent, species-specific, and interactive effects of these compounds within complex, mixed-species swards. These complexities limit our ability to intentionally design phytochemically optimized pastures. Another challenge is sustaining botanical and phytochemical diversity in grazed systems. Selective grazing, competition, soil fertility gradients, and climatic variability often reduce forb abundance, undermining long-term sward stability. Realizing the benefits of phytochemically enriched pastures therefore requires not only biological understanding, but also adaptive and novel grazing and management strategies that maintain diversity over time. This presentation will synthesize recent research on temperate forb species rich in bioactive compounds and their effects on animal performance, health, rumen function, and greenhouse gas mitigation, while also discussing practical strategies for integrating and sustaining these species in multifunctional pasture systems. These approaches contribute to multifunctional livestock systems capable of delivering environmental, production, and socio-economic benefits in rural landscapes.

Agroecological characterization of alpine pastures from visible to invisible: linking pasture use, vegetation, soil chemistry, and microbial communities.

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Alpine grasslands, classified as High Nature Value Farmland for their rich biodiversity, are vital agro-ecosystems supporting extensive livestock systems. This study investigates agroecological relationships in two alpine summer pastures in the Eastern Italian Alps: one grazed by sheep following three years of abandonment, and another historically grazed by dairy cows. During summers 2023 and 2024, we monitored grazing via GPS tracking to quantify pasture use intensity. Vegetation surveys using the Braun-Blanquet method identified multiple classes across both pastures. Topsoil samples were analysed for nitrate, ammonium, and organic carbon contents, as well as microbial communities via qPCR and sequencing. qPCR targeted nitrogen-cycle genes (nifH for fixation, amoA for nitrification, and nirK, nirS, and nosZ for denitrification) to assess functional potentials. Sequencing characterized community composition, with functional diversity inferred via FAPROTAX. In terms of pedological conditions, the nitrate:ammonium and organic carbon:nitrate ratios showed opposite trends and varied significantly across vegetation types and pasture-use intensities. These patterns were partly corroborated by distinct microbial community compositions and nitrogen/carbon-cycle functional potentials. Our findings highlight the interplay among pasture use, vegetation, and soil properties, including chemical status and microbial attributes, with key implications for nutrient cycling. This evidence supports agroecology-based strategies for sustainable management and conservation of alpine.

Integrating locally adapted legumes into multifunctional and extensive Mediterranean livestock systems

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Livestock farming systems in Mediterranean regions are increasingly challenged by climatic variability, feed market volatility, and environmental constraints. Strengthening feed autonomy and diversifying locally adapted forage resources are essential strategies for enhancing the resilience and multifunctionality of extensive livestock systems. In this context, alternative legume crops may play a key role in supporting sustainable and rural livestock production. Bitter vetch (Vicia ervilia), an ancient Mediterranean legume, represents a promising protein source for low-input and extensive livestock farming. Its tolerance to drought and marginal conditions, combined with favourable nutritional characteristics, makes it particularly suitable for small ruminant systems operating under grazing-based and mixed crop–livestock contexts. The aim of this study was to evaluate the nutritional value of six indigenous populations of bitter vetch and assess their potential contribution to resilient livestock feeding strategies. The trial was conducted under Mediterranean conditions (December 2018–June 2019). Seed and straw samples were analysed for dry matter (DM), crude protein (CP), neutral detergent fibre (NDF), and acid detergent fibre (ADF) to determine their suitability for ruminant diets and their potential role in reducing reliance on imported protein feeds. Bitter vetch seeds exhibited high crude protein concentrations (17.9–23.6% DM) and relatively low fibre fractions (NDF 24–30% DM; ADF 4.2–6.0% DM), indicating strong potential as a locally produced protein source. Straw contained moderate protein levels (6.2–9.7% DM) and fibre values (NDF 56.9–63.2% DM) consistent with its use as roughage in extensive feeding systems. The combined utilisation of seeds and straw allows flexible incorporation into ruminant rations, supporting both energy and protein supply under low-input conditions typical of Mediterranean grazing systems. Beyond its direct nutritional value, the integration of bitter vetch into crop–livestock systems may enhance multifunctionality at farm and landscape levels. As a legume, it contributes to biological nitrogen fixation, improving soil fertility and supporting soil health. Crop diversification through the inclusion of locally adapted legumes can reduce dependency on external feed inputs, strengthen system resilience under drought, and support biodiversity-friendly management of agricultural land. By reinforcing feed self-sufficiency and stabilising production under climatic stress, bitter vetch can contribute to the long-term viability of extensive livestock systems and the sustainability of rural territories. Its integration into Mediterranean farming systems represents a practical pathway towards resilient, multifunctional livestock production that supports ecological functioning while maintaining productive and socio-economic roles within rural landscapes.

Western Italian Alps are losing upland cheese production

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Upland dairy products are known for their specific features: the specificity of pasture botanical composition contributes to increase their polyunsaturated fatty acid content, and they express a diversified sensory profile, characterized by intense and floral notes. Their cheesemaking technology also largely varies across regions and relays on historical practices and local traditions, conferring to upland cheeses their uniqueness. However, mountain areas, especially in Western Italian Alps, are also characterized by considerable limitations in land-use, due to harsh climatic conditions, lack of infrastructures (roads, buildings) and services. Difficult working conditions, challenges in recruitment, wildlife conflicts, and insufficient generation renewal are menacing the maintenance of upland cheesemaking production. While a significant decrease in dairy production is widely perceived in mountain areas, quantitative data remain scares. The aim of this research was to take a census of upland active on farm cheesemaking plant in the Western Italian Alps and to compare data collected with the 2003 historical census aiming at quantifying trends of the last 20 years. We used a 425 km section of the Italian Alpine Club Walk "Sentiero Italia CAI" (crossing all the Alps) as a transect for the census. This transect crossed 15 valleys, and the whole Cuneo and half of the Torino provinces. Each upland pasture management unit (UPMU) crossed by the walk was identified and active on farm cheesemaking dairy plant were surveyed to characterise the farm, the herd and the cheesemaking production. Data form the same UPMU from the 2003 census were compared to those collected in 2025. On the total of 109 UPMU on the transect, 52 had an active cheesemaking plant in 2003, while only 35 remained active in 2025, with a reduction of 14.7%. Most of the remaining milk processing farms are accessible by roads and are equipped with barn and building. Here, the number of dairy cows milked on the farms was on average 33 vs. 36 in 2003. While total transhumant cattle numbers remained stable, there was an 8.0% increase in non-milking cattle (beef cattle, heifers, or dry cows). The total number of sheep and goats decreased of about -22% and -33%, respectively, mainly due to predation pressure and extra work needed for flock defence. The workforce on cheesemaking farms (mainly farmer's relatives) was 23% higher in 2025 than in 2003, probably because of the extra workforce demanded for modern farm management and for herds defence from wolf attacks. Cheese production on the UPMUs with an active cheesemaking plants decreased of about -7.3%, but when considering also the missed cheese produced in 2003 on those upland pasture management unit that stopped milk processing, the decrease was much consistent (-36.4%). In conclusion, even if the presented data are preliminary and partial, it is evident that Western Italian Alps lost about one third of the upland cheese in the last 20 years. The remaining cheesemaking farms on upland are those with adequate infrastructure and with large families capable of providing the required workforce. Urgent policy intervention is needed to invest in infrastructures (roads ad buildings), and facilitate employment to preserve upland cheesemaking production.

Added value of grassland based dairy farming systems: the Trentingrana PDO production in Eastern Italian Alps.

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Mountain grassland-based dairy livestock farming is associated with the provision of several positive environmental externalities, also ensuring the management of marginal pastures and supporting local tourism activities. These systems are normally characterised by a stronger link with the territory when compared to conventional ones located in plain areas, contributing to support the local economy. Therefore, specific indicators are needed to maximise the positive contributions of these systems and mitigate the negative ones. This study aims at investigating mountain grassland-based dairy farming, to develop tailored indicators to maintain its link with the territory and its positive associated externalities. Farm structure and management data were collected from 23 grassland-based dairy farms under the Trentingrana PDO cheese certification scheme, located in three mountain areas (Val di Fassa, Primiero, Val di Non) in the province of Trento (Italy). A Principal Component Analysis, conducted to select the most representative variables, identified 4 indicators: altitude, farmland area, share of permanent grasslands (PG), loose housing. These indicators were combined with farm locations data to perform a K-means clustering, obtaining 3 clusters. Additional indicators for geo-morphological and landscape features and ecosystem services were computed using the software QGIS. A one-way Anova was then performed to investigate differences between the clusters. Cluster 1 represented mid-altitude farms (961m asl), with medium surfaces (35 ha), the largest herds (75 cows), total share of loose housing, the highest milk yield (11715 kg Energy Corrected Milk – ECM – /cow/year), the lowest field slope (8.66°) and no agrotourism activity. Cluster 2 described mid-altitude (802 m asl), smallest farms (22ha and 24 cows), with farm surfaces totally managed as permanent grasslands, medium share of loose housing (50%), the lowest milk yield (8088 kg ECM/cow/year), medium field slope (10.81°) and 44% of farms with agrotourism activity. Cluster 3 referred to farms located at the highest altitude (1523 m asl), managing the widest surfaces (69 ha), with medium-sized herds (45 cows), the lowest number of cuts on PG (1.7), farm surfaces totally managed as permanent grasslands, medium share of loose housing (65%), medium milk yields (10087 kg ECM/cow/year), the highest field slope (13.64°) and 67% of farms with agrotourism activity. Clusters were similar for the other factors considered, including milk price and additional ecosystem services indicators. In conclusion, farms belonging to Cluster 1 were reasonably located in more favourable areas, adopting a higher level of intensification carried out with increased herd size and milk yields, but without diversifying their activity through agrotourism. Farms belonging to Cluster 3 were situated in less favourable areas, located at the highest altitude and with fields characterised by the highest slope, but were able to maintain high production levels, also diversifying their activity by integrating agrotourism to a certain extent. Farms grouped into Cluster 2, despite being located in favourable areas, managed the smallest surfaces and herds, obtaining the lowest production levels and still adopting a medium share of tie-stall housing, therefore maintaining a more traditional production system. The above-mentioned indicators will be integrated with impact categories (Life Cycle Assessment and nutrients excretion data) and ecosystem services indicators, to monitor the multiple relationships between dairy farming and agroecosystems in the Trentingrana PDO cheese production chain.

Site-adapted farming systems in mountain regions: a synthesis of key characteristics, transformations and challenges

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The diversity of environmental conditions in mountain regions has created a large diversity of locally adapted farming systems. Common to many systems are the challenges in harvesting and transporting forage, which favours grazing over mowing and transhumance over stationarity. Moreover, communal ownership is frequent to use labour and infrastructure more efficiently. Recent decades have seen important transformations of demographic and agricultural structures in European mountain regions. In general, the number of people working in agriculture decreased while farm sizes increased. Managing larger units and more land requires investments into infrastructure and machinery. These investments, in turn, require the specialization of animal production and of forage conservation as well as a rationalization of work processes by mechanization. The professionalization and rationalization of farm operations potentially decouple farming systems from the local conditions: Forage production is concentrated on flat and productive sites, manageable with machinery. Highly productive livestock allows higher output at lower number of heads but is less suited to graze low-productive sites and is thus supplemented with concentrates or herded on the best sites only. Summarizing a series of recent own studies we identify key elements of site-adapted farming systems in mountain conditions: (1) livestock diversity and productivity aligned with the diversity of environmental conditions and the productivity of available farm land, (2) closed nutrient cycles and circular flows to prevent the degradation of the natural grassland ressources, and (3) non-agricultural activities to valorize non-provisioning ecosystem services produced by farming. We illustrate these key elements with examples from the Alps and other mountain ranges.

Lamb meat production in marginal areas: a comprehensive carbon footprint assessment accounting for soil carbon sequestration

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In much of the Mediterranean basin, sheep farming is predominantly oriented towards dairy production, with ewes selectively bred to enhance milk yield. However, in certain marginal areas of southern Italy, such as the Basilicata region, sheep husbandry is mainly practiced for lamb meat production. Against this backdrop, the present study aimed to evaluate the environmental performance of lamb meat production in marginal areas, focusing on the carbon footprint (CF) and explicitly accounting for carbon sequestration. Five lamb meat farms, representative of the reference geographical area, were selected for the analysis. All farms are managed under extensive production systems, characterized by a wide reliance on grazing resources, limited use of external energy inputs, and the complete absence of both organic and inorganic fertilizers. The main breeds reared are the Gentile di Puglia sheep and the local crossbred. On average, the farms cover 167 ± 44 hectares, with 218 ± 32 adult ewes per farm. Annual lamb sales amount to 192 ± 21 head, with an average live weight (LW) of 18 ± 1 kg. The CF was quantified using a Life Cycle Assessment approach in accordance with ISO 14040-44 standards, applying a cradle-to-farm-gate system boundary. Emissions were expressed as kg CO_2 equivalent and calculated using the open-source software openLCA (v.2.6), supported by the AGRIBALYSE and Ecoinvent databases. The Environmental Product Declaration (EPD 2018) impact assessment method was used. Two functional units were considered: LW (kg) and land area (ha). Soil carbon sequestration (SCS) was estimated using the crop residue approach and subsequently deducted from the overall CF. The average of CF amounted to 14.2 ± 3.3 kg CO_2 eq per kg of LW and 452 ± 111 kg CO_2 eq per ha (mean ± SD). When SCS was included in the accounting framework, the overall environmental impact decreased by 30%. The main contributors to total emissions were biogenic CH_4 (64%), followed by N_2O (29%) and fossil CO_2 (7%). Overall, the findings highlight the central role of enteric methane in determining the CF of extensive lamb production systems and demonstrate the substantial mitigation potential associated with SCS in grazing-based farms. Properly accounting for soil carbon dynamics is therefore essential to avoid overestimating the climate impact of low-input, pasture-based systems. In addition, the relatively low CF observed per unit of land area reflects the limited external inputs and the extensive nature of these production systems, confirming their low environmental intensity at the regional level. This aspect is particularly relevant in marginal areas, where sustainable livestock management can contribute not only to food production but also to landscape conservation and the provision of various externalities and ecosystem services. Overall, the study provides a first benchmark for lamb meat production in Southern Italian marginal areas and supports the environmental value of extensive sheep farming when its multifunctional role is fully acknowledged.

Effect of breed on the health and welfare of semi-extensively reared dairy goats in Greece

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Dairy goat farming plays a vital socioeconomic role in marginal regions, though sustainability is often hindered by animal health and welfare challenges in semi-extensive farming systems. The objective here was to assess the effect of breed on health and welfare traits of semi-extensively reared dairy goats in Greece. A total of 1,210 goats from seven farms were used. Goats belonged to Eghoria (n=418), Skopelos (n=429), and Damascus (n=363) breeds. Health and welfare traits of individual goats were recorded monthly across two consecutive milking periods. Health records included milk somatic cell count (SCC), total viable count (TVC), and parasitism with gastrointestinal nematodes (GIN). Welfare records included body condition score (BCS), horn injuries, ocular and nasal discharge, head injuries and lesions (collectively analysed as head problems), skin injuries and lesions, body and udder abscesses, udder asymmetry, and overgrown claws. Breed effects on the studied traits were analysed using mixed linear and non-linear models in R. In addition to breed, models included the fixed effects of period of kidding, age at kidding and days from kidding, and the random effects of animal and farm. Significant breed differences ($P<0.05$) were identified for BCS, udder asymmetry, and head problems. Specifically, Skopelos goats had higher BCS compared to Eghoria and Damascus goats. The odds for udder asymmetry were three and two times higher for Eghoria and Damascus, respectively, than Skopelos goats. Head problems were ten-times more likely in Damascus and Eghoria relatively to Skopelos goats. These results highlight significant breed-specific differences in dairy goat welfare traits, and a comparatively better welfare status in Skopelos goats, likely reflecting their better adaptation to semi-extensive farming conditions. Collection of data was funded by the European Commission Seventh Framework Program (FP7 Food, Agriculture and Fisheries, Biotechnology) for the project Sustainable Organic and Low Input Dairying (SOLID), grant number FP7-KBBE-266367.

Adoption of regenerative management practices in Mediterranean Dehesa Livestock Systems and their contribution to ecosystem services

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Mediterranean Dehesa Systems are multifunctional extensive livestock systems based on the sustainable use of pastureland, trees and natural resources. They play an important role in biodiversity conservation, wildfire prevention, climate regulation and rural employment. Regenerative management practices may further enhance their environmental performance and resilience. However, the degree to which these farms are implementing regenerative techniques remains unclear. The objective of this study was to evaluate the adoption level of regenerative practices in dehesa livestock farms and to assess their potential contribution to ecosystem services. A survey was conducted on 52 livestock farms in Extremadura (Spain) during 2021–2022. A structured questionnaire collected information on soil management, organic matter cycling, grazing strategies, tree management and biodiversity-related actions. Adoption rates were analysed descriptively to identify patterns across management domains. Results show a coexistence of traditional and conventional practices, with limited implementation of specific regenerative techniques. In organic matter management, 53.8% of farms use pruning residues for browsing, whereas composting (3.8%) and biochar production (0%) are almost absent. Additionally, 19.2% still burn residues. Conventional tillage is used by 19.2% of farms, while minimum tillage (9.6%) and no-till (3.8%) remain marginal. Although 53.8% practice pasture rotation, 75% rely on large fixed paddocks, and adaptive grazing tools such as mobile fencing (11.5%) and electric fencing (17.3%) are underused. Regarding ecosystem services, 61.5% of farms use grazing for wildfire prevention, highlighting their contribution to landscape management. Tree planting (30.8%) and beekeeping (42.3%) indicate diversification strategies linked to biodiversity support. However, ecological infrastructures such as hedgerows and corridors are scarce (7.7–9.6%). Furthermore, 30.8% of farms report severe erosion, yet corrective measures are rarely implemented. Dehesa livestock systems maintain a strong ecological base and already provide relevant ecosystem services. Nevertheless, the structured adoption of regenerative soil and grazing management remains limited. Enhancing technical training and knowledge transfer could strengthen system resilience, improve environmental outcomes and reinforce the role of extensive livestock farming in sustainable rural development.

Multifunctional extensive small ruminant farming in Amorgos island (Greece): implications for rural development and ecosystem services

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Extensive small ruminant farming systems in Mediterranean insular regions represent multifunctional production models that simultaneously sustain rural livelihoods and deliver essential ecosystem services. This study assesses the structural, productive and environmental characteristics of sheep and goat farming in Amorgos Island (Greece), within the framework of a rational livestock management plan. Primary data were collected through structured interviews with 16 representative farms, complemented by genomic analysis of indigenous sheep, official livestock registry and arable crop statistics. The system is predominantly extensive (87.5%), based on year-round utilizing rough grazing on steep slope rocky hills as well as flat land cultivated parcels by sheep and goats. Local breeds dominate, selected mainly for environmental adaptability and resilience under low-input conditions. Reproductive performance is satisfactory (fertility ~83–84%), adult mortality remains below 5%, and animals exhibit extended productive longevity, reflecting adaptation to local constraints. Beyond food production, the system provides other significant ecosystem services. Continuous grazing contributes to shrub biomass control and wildfire risk mitigation, while manure deposition enhances soil fertility in both rangelands and small arable plots. The preservation of autochthonous genetic resources strengthens system's drought tolerance and climate resilience. Moreover, grazing maintains heterogeneous agro-pastoral landscapes that support biodiversity conservation. Economically, livestock production is closely linked to local tourism and short supply chains, with a high proportion of meat and dairy products marketed directly or through family businesses. However, challenges include ageing farmers, limited succession, drought-induced pasture decline and reliance on purchased feed. Overall, the Amorgos case demonstrates how multifunctional extensive livestock systems can reinforce rural cohesion, prevent land abandonment and enhance ecosystem services in Mediterranean marginal environments.

Effects of Pasture Diversification Strategies on Milk Production in Dairy Cows

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Pasture diversification is increasingly promoted because of its agronomic, nutritional, and environmental benefits. However, establishing and maintaining pasture diversity remains challenging because of edaphic and environmental constraints, as well as the generally lower persistence of forbs relative to grasses. Diversification through mixed cover crops and herbal leys may provide a practical strategy to enhance pasture diversity and improve animal performance. This study evaluated the effects of pasture diversification through the inclusion of forbs alone or in combination with herbal leys on milk yield, milk composition, nitrogen partitioning, and methane emissions of grazing dairy cows during spring 2026. Pastures were established in a randomized complete block design with four replicates. Thirty-six mid-lactation Jersey cows (19 primiparous and 17 multiparous) blocked for parity, body weight, and milk yield grazed one of three pasture systems: (1) simple pasture, (2) diverse pasture, or (3) diverse pasture supplemented with herbal leys. Diversification through the inclusion of forbs alone was limited because of substantial volunteer annual ryegrass invasion; however, the addition of herbal leys increased forb content in the pastures to 37% as compared to 14% in simple and 19% in diverse pastures. Cows grazing the diverse + herbal ley treatment produced numerically greater ($P = 0.11$) milk yield (16.3 L/d) compared with cows grazing the simple (14.8 L/d) and diverse pastures (15.2 L/d). Milk composition was not affected by pasture treatments (all $P > 0.05$). Measurements related to environmental outcomes, including enteric methane emissions, and animal health parameters are currently being analyzed. Summer and fall grazing evaluations will also be conducted in 2026 to determine the influence of seasonal variation in pasture conditions. Overall, these preliminary findings suggest that incorporating herbal leys can successfully increase forb abundance and may enhance milk production in pasture-based dairy systems, even when conventional diversification approaches are constrained by grass competition and climatic conditions.

Towards a New Selection Index for Holstein Cattle in Pasture-Based Production Systems: Stakeholder Perspectives from a Survey

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Pasture-based dairy cow management systems are associated with animal welfare and resource efficiency. However, the number of such farms in Germany has declined in recent years, despite increasing societal and political expectations. Currently, no national selection index explicitly reflects the breeding priorities of farmers operating pasture-based dairy systems. To address this gap, a nationwide survey was conducted to assess the role of grazing in German dairy farming, identify key challenges and explore stakeholder perspectives on breeding goals relevant to grazing systems. Using an online survey, dairy farmers received questions regarding socio-demographic characteristics, farm structure, grazing management, motivations for or against grazing, challenges associated with grazing and their ideas concerning breeding of Holstein Friesian (HF) cows suitable for pasture-based production systems. The questionnaire included multiple choice items, Likert-type scales and adaptive question paths. Responses from 329 participants collected between March 2025 and January 2026 were used for a preliminary descriptive analysis. Among respondents, 84 % practiced grazing, with most farms located in Northern Germany. HF was the predominant breed, and the average herd size was slightly higher than the national average of animals per herd. Most farms offered more than six hours of daily pasture access to lactating cows, while full grazing without supplementation (4 %) was rare. Rotational grazing systems were most common, only young stock was mainly kept on full pasture. The main reasons for grazing were animal health aspects (95 %) and personal conviction (91 %), followed by reputation (71 %). External drivers such as certification (34 %) or milk price premiums (43 %) seem to play a minor role. In contrast, farms without grazing primarily reported economic disadvantages (83 %), increased labor (77 %) and a lack of pasture close to the farm (75 %) as main barriers. Farmers with grazing cows were asked whether they see merit in the development of a new breeding value specifically designed to fit the needs of farms with grazing cows, and nearly 50 % expressed interest. Using a participatory approach, HF farmers with an interest in breeding who had their own ideas about pasture genetics (n = 20) as well as experts from breeding organizations (n = 10) were asked to evaluate the relative importance of various traits for HF kept on pasture. In addition to traits included in the German total merit index (RZG), body condition score and feed efficiency should also be considered. Furthermore, not only the relative breeding value for milk, but also those for milk components were of interest. Based on the survey conducted among farmers, milk traits (milk yield: 17.3 %; milk components: 11.2 %) received the highest weighting, followed by longevity (23.1 %), feed efficiency (11.7 %) and conformation (10.9 %). Although the indices designed by the individual farmers varied to some extent, the overall index was similar to expert assessments but differed from the RZG, particularly in the emphasis placed on milk production traits and health. The study indicated a demand for the development of a specifically tailored selection index for pasture-based dairy production as it is already established in other countries.

Emerging practices and perceptions of regenerative agriculture across pioneering Dutch dairy farms

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Dutch agriculture, and particularly the dairy sector, is confronted with several sustainability challenges regarding biodiversity, soil quality and nitrogen excretion. A transition towards more regenerative agriculture (RA) has been suggested as one way to address these challenges. However, there is no generally accepted scientific definition of RA yet. Therefore, this study aims to identify the characteristics and applied practices of regenerative dairy farming and how this compares to the general dairy production practices in the Netherlands. Interviews with 28 RA pioneering farms were carried out. These farms voluntarily participated in a large research programme (ReGeNL) on supporting RA in the Netherlands. They are applying (combinations of) innovative practices that distinguish them from the conventional Dutch dairy farms and have the potential to improve their regenerative capacity. Examples of such practices are grazing and feed extensification, technical innovations such as a mobile pasture milking robot, integration of agroforestry and shortening of value chains. Two rounds of interviews were carried out. The first round aimed to get a general understanding of the farm system (e.g., size of the operation, origin story of the farm, general distribution of production) and their perception of RA (e.g., why did they start with RA on their farm?; what themes are most relevant to them now and in the future?). The second round consisted of getting a detailed dairy cow management overview (e.g., calf weaning process, feed and grazing management, medical intervention plan). Preliminary analysis of the first-round interviews showed an average farm size of 98.8 ha (41 – 200 ha) with 88 lactating dairy cows (30 – 230 cows), where the average Dutch dairy farm cultivates 60 ha and milks 119 dairy cows. Additionally, the farmers' perspective on RA mainly comprised the improvement of natural closed cycles on farm, which often coincided with minimizing reliance on external inputs. The theme deemed most important in moving towards RA was soil health (for its regulatory and cycling capacity) followed by the theme animal (in relation to health, production and welfare). Overall jointly listed as third by the farmers are the themes economy and biodiversity. The preliminarily results of the second interview round showed strong variation across pioneering farms. For example all farms practice grazing, but the types varied from rapid rotational strip grazing to kurzrasen, with variable occupation ranging from 2 to over 100 animals/ha on occasion. Grazing strategy varied throughout the season depending on grass availability. Total grazing duration varied between farms ranging from 720 h (the minimum to label milk as pasture-based) to 4200 h annually. Whereas in the Netherlands, about 80% of dairy farms implement grazing, reaching an average annual grazing duration of 1301 hours. Furthermore, diet composition varied strongly between the interviewed farms, for example concentrate provision ranged from 0 kg (on a purely grass-based farm) to 30 kg per 100 kg milk yield (Dutch average: 30.5 kg). Finally, the breed composition on these farms varies substantial resulting in herds of up to 6 different breeds, with only about half of the farms having Holstein cows as one of the main breeds in the herd, where the overall Dutch herd stock consists for 90% of Holstein Friesian. In conclusion, RA dairy farms displayed distinct characteristics compared to the average Dutch dairy farm, most notably a shared tendency towards extensification. Nevertheless, considerable variation existed in the approaches of individual farms, which was linked to differences in available land, knowledge, and financial resources.

The potential of grass biorefinery on dairy farms

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The dairy sector is under increasing pressure to close nutrient cycles, reduce environmental impact, and develop innovative on-farm revenue models. Biorefinery of grass might offer a promising solution. Grass refinery is a physical process in which fresh grass is pressed and separated in two fractions. A liquid fraction (press juice), which is rich in protein and can be used in feeds for monogastrics or in human food products. And a fibrous fraction, which can be ensiled (press cake) and remains on the dairy farm, or can be valorised in other sectors like construction or the paper industry. By replacing part of the conventional grass silage in the ration with press cake from biorefined grass, both nitrogen (N) intake and N excretion can be reduced and N efficiency can be increased, without compromising animal performance. Grass biorefining is technically available, but its practical implications for closing nutrient cycles on dairy farms and farm economics have hardly been investigated or quantified. The objective of this study was to assess the effects of grass biorefining on nutrient cycling and the economic performance of dairy farms for three farm types. The effects of grass biorefining were assessed with the DairyWise model and the Annual Nutrient Cycle Assessment (ANCA) model for three typical Dutch dairy farms: an intensive dairy farm on clay soil (C farm) and two more extensive dairy farms on peat (P farm) and sandy soils (S farm). The initial situation was compared with a scenario in which grass biorefining is applied on five hectares of grassland per farm. The impacts on farm management, economic results, and sustainability indicators were assessed. The model analysis indicated that related to farm management the application of grass biorefining affects the amount of available roughage, because of dry matter allocation in the press juice. On the other hand, the refinery process improved the digestibility of the press cake, resulting in a favorable ileal digestible protein level and a reduced rumen degradable protein balance. To compensate for the loss of roughage, the C farm bought more maize silage where the extensive P and S farm sold less surplus of grass silage. Grass biorefining reduced the amount of concentrates per cow per year, with 39, 11, and 68 kg for the C, P and S farm, respectively. However, especially for the C farm more protein rich concentrates were needed. Regarding fertilization, grass refining showed both positive and negative effects on the required input and composition of mineral fertilizers, depending on the farm situation. From an economic perspective, applying grass biorefining on five hectares of grassland resulted in a positive balance of € 810, € 560 and € 750 per hectarefor the C, P and S farm respectively. The positive effect of grass biorefinement on farm income came from compensation for land use and the press juice, reduced contractor costs and manure disposal costs, although this might be partly offset by decreased roughage sales and increased storage needs, again, depending on the farm situation. For sustainability indicators, grass biorefinery led to a reduction in ammonia and greenhouse gas emissions (methane and nitrous oxide). With grass refinery, ammonia emissions reduced 2, 4 and 3% for the C, P, and S farm, respectively. This modeling study provided insights into the practical implications and key considerations of grass biorefinement on dairy farms. Validation under real farm conditions is required to determine how these model results translate into farm-specific constraints, practices, and results. In conclusion, grass biorefining is a promising strategy to enhance nutrient efficiency and farm profitability.

Winter grazing by dairy cows: a viable solution for climate change adaptation

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With climate change, grass growth in western France is expected to increase in late autumn and late winter, periods when soil bearing capacity is not always sufficient for grazing. This can lead to a large stock of grass biomass which, if not utilized before spring, reduces pasture quality. This study aims to assess whether winter grazing by dairy cows could be a suitable solution for utilizing this grass. It also seeks to determine whether this forage could help improve winter rations in organic farming systems, which are often deficient in protein due to the lack of satisfactory solutions for on-farm production of protein concentrates. Trials conducted over three winters (2022–2025) at the Trévarez organic experimental farm (Western France, Chamber of Agriculture of Brittany and Idele) aimed to characterize winter grass and evaluate the impact of winter grazing on pastures (growth, composition, potential degradation, trampling). The results showed that the median grass density was 245 kg Dry Matter (DM) ha^{-1} cm^{-1}. Average growth was 4 kg DM ha^{-1} day^{-1}, corresponding to 360 kg DM ha^{-1} accumulated over three months (mid-November to mid-February). Winter grass consisted of more than 80% grasses at the leafy stage, which explains its good nitrogen value (20–22% crude protein in DM, with an average DM content of 15%). Winter grazing did not reduce annual yield but delayed the spring regrowth of the pasture by about two weeks. The experiment also studied the response of a lactating herd grazing grass–legume pastures during winter as a complement to an indoor ration consisting of ad libitum grass silage and 5 kg DM of maize silage. An experimental group of 25 lactating cows grazed for 3 hours per day, in two grazing rotations, during the three winters between December and February. The performances were analysed with an ANOVA model with R Studio software (tests with $\alpha = 5\%$). With grazed grass intake estimated using the Inrae HerbValo method at 2.6 kg DM cow^{-1} day^{-1}, these cows produced on average significantly 1.8 kg cow^{-1} day^{-1} more milk for two winters out of three (no significant effect at $p<0,05$ during the third winter) than the control group that remained indoors on the same basal ration. This was associated with a reduction in grass silage intake of 1 kg DM $cow^{-1}day^{-1}$. Overall intake increased and the nitrogen balance of the ration improved slightly: 85 vs. 80 g PDI per UFL (Intestine Digestible Protein per Energy Unit, INRAe 2018) for the non-grazing control group. No effects were observed on milk components, body condition, or animal health. From an economic perspective, 3 hours of grazing for a herd of 75 dairy cows would increase the margin over feed costs by about €2,100 per month in two winters out of three. Winter grazing may therefore be a way to better balance the protein supply of winter rations, particularly in organic dairy farming. However, this practice requires well-designed farm tracks and must be adapted to pedo-climatic conditions (stocking rate and grazing duration adjusted to soil bearing capacity and cumulative rainfall). Winter grazing could thus represent an opportunity to utilize the grass available in late autumn and make better use of the effects of climate change, especially in livestock regions with an oceanic climate.

Adapting Dairy Cattle Grazing Systems to Climate Change: The Role of Agroforestry

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Climate change alters the thermal environment of grazing animals, affecting their productivity, health, reproduction, and feed conversion efficiency. In addition, the quality and quantity of forage in grassland systems are strongly influenced by the increasing frequency of extreme weather events. Consequently, targeted strategies in pasture and grazing management, supplementary feeding, and the use of alternative forage crops are needed to address the challenges posed by climate change. Agroforestry represents a land-use strategy that can help mitigate climate change while providing environmental, economic, and social benefits. The literature indicates that agroforestry systems may alleviate heat stress by improving microclimatic conditions and enhancing thermal comfort for grazing animals. However, factors such as tree canopy and trunk density, the arrangement and orientation of tree rows, and the density of scattered trees must be carefully considered in system design, as they influence heat stress risk by modifying solar radiation, air temperature, humidity, and wind circulation. In agroforestry systems, herbage biomass is primarily reduced due to limited light availability and, to a lesser extent, competition for nutrients. However, effects on nutritive value differ between grasses and legumes. Under shaded conditions, crude protein content tends to increase in grasses but decrease in legumes, while fibre content generally remains stable or declines. Grass–legume mixtures can enhance both yield and forage quality through complementary species interactions. Adopting optimal grazing management that accounts for shade, forage yield, and nutritive value is therefore essential to improve animal productivity. Agroforestry systems can also provide tree fodder to mitigate the effects of summer drought and heatwaves on herbaceous forage. Dairy cows can consume leaves and young shoots less than 8 mm in diameter, either directly from the tree or through branch foddering. Feeding livestock with leaves and shoots harvested from species such as maples (Acer spp.), ashes (Fraxinus spp.), lindens (Tilia spp.), elms (Ulmus spp.), poplar (Populus spp.) and white mulberry (Morus alba) is a traditional practice in several European regions. Given the increasing frequency of droughts and heatwaves that negatively affect forage yield and quality, the contribution of forage trees could become strategically important in counteracting the effects of climate change. The nutritional value of tree foliage is comparable to, and often higher than, that of many herbaceous forage species. Moreover, in many species, tree leaves contain significant levels of tannins, polyphenols, and other secondary metabolites that may help reduce gastrointestinal parasite burdens and mitigate methane emissions. Overall, integrating trees with forage crops or grasslands in agroforestry systems represents a promising strategy to address climate change challenges, particularly those related to drought and heatwaves.

Herbage mass and pasture allowance affect grazing behavior more than milk production in dairy cows grazing polyphytic pasture in southern Chile

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This study evaluated the effects of herbage mass (HM) and pasture allowance (PA) on milk production, dry matter intake (DMI), and grazing behavior of dairy cows grazing a polyphytic pasture during autumn in southern Chile. The pasture was mainly composed of Lolium perenne L., Trifolium repens L., and Bromus valdivianus Phil. The experiment was conducted at the Dairy Research Station of Universidad Austral de Chile (UACh), Valdivia, Los Ríos Region, Chile, during April and May 2025 (61 days), using rotational strip grazing across three paddocks with a total area of 9 ha. Grazing was delayed from late February to achieve the target HM. The basal diet consisted of polyphytic pasture supplemented with pasture silage (5.0 kg DM/cow/day, offered immediately after afternoon milking), commercial concentrate (4.5 kg DM/cow/day, offered in two equal portions during milking), rolled corn (1.8 kg DM/cow/day), mineral salts (0.25 kg DM/cow/day), and water ad libitum. Thirty-six clinically healthy Holstein Friesian dairy cows (28 multiparous and 8 primiparous), with somatic cell counts <200,000 cells/mL, milk production of 26.9 ± 1.25 L/day, days in milk (DIM) of 38.0 ± 4.17, and average body weight (BW) of 530 ± 4.9 kg, were assigned to one of four treatments in a 2 × 2 factorial arrangement: two HM levels [2,200 kg DM ha−1 (standard HM, SHM) or 3,000 kg DM ha−1 (high HM, HHM)] and two PA levels [17 kg DM cow−1 day−1 (low PA, LPA) or 25 kg DM cow−1 day−1 (high PA, HPA)], with nine cows per treatment.The variables evaluated included milk yield recorded at each milking throughout the experiment; milk fat, protein, and urea concentrations analyzed during the last week of each month from three consecutive milkings; grazing behavior recorded over a 24-h period on two occasions; and DMI determined during weeks 3 and 7 of the experiment. Data were analyzed using a generalized linear model. No HM × PA interaction was detected for milk yield (25.9 ± 1.23 kg cow−1 day−1) or milk protein concentration (3.78 ± 0.06%; $p > 0.05$). However, milk fat concentration showed a significant interaction ($p < 0.05$), with SHM_HPA showing the highest value (4.35 ± 0.97%).Pasture DMI differed according to PA level, with cows under HPA consuming 5.7 kg DM animal−1 day−1 compared with 3.9 kg DM animal−1 day−1 under LPA (SEM = 0.264). Total DMI was also greater under HPA than under LPA (17.6 vs. 16.0 kg DM cow−1 day−1; SEM = 0.36). Grazing time and idling time were affected by the HM × PA interaction ($p < 0.05$), whereas rumination time was not. The main effects showed differences only for HM in grazing time (407 vs. 370 min for SHM and HHM, respectively) and time spent on other activities (469 vs. 531 min for SHM and HHM, respectively). Although HM and PA had limited effects on milk yield and milk composition, these management factors influenced grazing behavior and pasture intake, indicating that cows adjusted their grazing activity in response to pasture availability rather than increasing milk output. Project financed by FONDECYT 1240451.

Milk urea concentration ranges suitable as indicators of nitrogen utilization in grazing dairy cattle

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Bulk milk urea (MU) concentration is widely used as an indicator tool to support diet formulation, improve nitrogen (N) utilization and reduce surplus urinary nitrogen (UN) excretion from dairy cattle managed under confinement systems. However, there is limited information on appropriate use of MU concentration ranges under pasture-based systems. This study used three datasets to determine lower and upper bulk MU concentrations in pasture-fed dairy cows: 1) contemporaneous (i.e. group mean) measurements of individual cow MU concentration and UN excretion from a cow phenotyping study (n=131); 2) measurements of herd bulk MU concentration from a 1-year observational study including four visits to 38 commercial farms in New Zealand (n=150); and 3) treatment means of individual cow MU concentration and UN excretion from a meta-analysis of 26 publications (n=76). Specific data available, animal management and measurement approaches varied across and within datasets, enabling identification of consistent trends across relevant, diverse contexts. Milk urea (mg/dL) was analysed by spectral or enzymatic methods, with UN excretion (g N/d or % N intake) estimated by urine spot sampling, sensors or total daily collection. Dietary energy and protein requirement and supply variables were estimated using milk production, live weight, feed composition and calculated or measured DM intakes. Relationships between MU concentration, UN excretion and variables that indicate either a deficit, a balance (where animal N requirements are met), or a surplus of dietary protein in lactating dairy cows were examined for each dataset. Pairwise correlation analyses (CORR procedure) to estimate linear Pearson (r) and non-linear monotonic Spearman (rs) correlation coefficients were performed in SAS (v9.4). Deciles of MU data were obtained (UNIVARIATE procedure in SAS), and variables of interest plotted and compared across deciles using one-way ANOVA and Tukey HSD testing in R (v4.2.0). Results indicated that N supply parameters, including dietary crude protein concentration, rumen degradable protein (RDP) balance and metabolizable protein (MP) balance, were positively correlated with MU concentration and UN excretion. By estimating the supply of RDP and MP relative to the animal requirements, we obtained a range of MU concentrations between 17 to 22 mg/dL as indicative of dietary N supply being close to animal requirements (i.e. balance); however, there was no consistent negative association between MU concentration below this range and milk production parameters. Thus, low MU values should be interpreted cautiously and not assumed to reflect dietary protein deficiency on their own. Increases in MU concentrations were linked with increases in daily UN excretion (expressed as g/N, % N intake, or relative to milk N output) and decreases in N use efficiency (milk N output/N intake). Milk urea concentrations exceeding the range of ~25-33 mg/dL were indicative of dietary N supply exceeding cow requirements and therefore increasing the likelihood of higher UN excretion. However, high variability in the datasets limited the development of predictive equations for UN losses based on MU concentration. Bulk MU can be used alongside other information to identify opportunities to optimize pasture and supplementary feed management, improve N use efficiency and reduce environmental risks of N losses from grazing dairy systems.

Effect of increasing lucerne-to-maize silage proportion in diets on performance of dairy cows

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The use of lucerne silage in dairy cow feeding systems has regained interest due to its numerous agronomic and zootechnical advantages. Combining lucerne silage with maize silage appears to be a promising feeding strategy, while using less protein-rich concentrate and increasing protein self-sufficiency. However, interactions between the two forages may influence feed intake and the nutritional value of the diet, and incorporation rate of lucerne silage in the diet may affect these interactions. This study aimed to evaluate the effect of 3 different proportions of lucerne and maize silages (25:75, 50:50, and 75:25; referred to as L25, L50, and L75, respectively) in forage-only based diets on voluntary dry matter intake (DMI), milk production, and enteric methane emissions in dairy cows. A total of 24 mid-lactation Holstein cows were assigned to a double 3 × 3 Latin square design with three treatments and three consecutive periods of 4 weeks. The DMI was lower on L25 (16.9 ± 0.39 kg/d, mean ± SE; $P < 0.001$) than on L50 (18.8 ± 0.39 kg/d) and L75 (19.1 ± 0.39 kg/d), probably partly due to the lower crude protein content of the L25 diet (93 vs 139 g CP/kg DM on average). Cows fed L50 produced more milk (20.4 ± 0.57 kg/d; $P < 0.05$) than those fed L25 (19.3 ± 0.57 kg/d), while L75 showed an intermediate value (19.9 ± 0.57 kg/d). A similar pattern was observed for energy-corrected milk yield ($P < 0.05$), which was higher with L50 (20.0 ± 0.51 kg/d) compared with L25 (18.8 ± 0.51 kg/d) and L75 (19.2 ± 0.50 kg/d). Milk fat and protein contents did not differ among treatments, averaging 39.0 ± 1.12 g/kg for fat ($P = 0.12$) and 29.5 ± 0.44 g/kg for protein ($P = 0.06$). In contrast, milk fat yield was higher for L50 (800 ± 23.2 g/d; $P = 0.02$) than L25 (750 ± 23.2 g/d) and L75 (764 ± 23.1 g/d), and protein yield was greater for L50 (608 ± 16.8 g/d; $P = 0.04$) compared with L25 (569 ± 16.8 g/d), L75 being intermediate (584 ± 16.7 g/d). Daily methane production tended to be higher for L50 (419 ± 11.1 g/d) than for L25 (414 ± 11.1 g/d) and L75 (398 ± 11.0 g/d; $P = 0.051$). Methane production per kg of DMI decreased from 23.7 ± 0.55 g/kg for L25 to 21.5 ± 0.55 g/kg for L50 and 20.3 ± 0.55 g/kg for L75 ($P < 0.001$). Methane emissions per kg of milk was highest in L25 (21.8 ± 0.57 g/kg) and lowest in L75 (20.1 ± 0.56 g/kg), with intermediate values in L50 (20.8 ± 0.57 g/kg; $P < 0.05$). Overall, this study confirmed interactions between the two forages, with the L50 providing the most favorable balance by ensuring higher feed intake and milk production while reducing methane emissions, whereas increasing lucerne to 75% offered no additional production benefits. These results highlight the potential of optimising forage composition of mixtures to enhance both productivity and environmental sustainability.

Co-ensiling whole crop corn with increasing percentages of common beans (Phaseolus vulgaris)

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Growing corn together with crops that are more attractive to insects, particularly pollinators, is discussed as measure to improve the impact of corn silage production on insect biodiversity. Common beans (Phaseolus vulgaris) are relatively well-suited to be cropped together with corn for that purpose, as common beans (CB) are very attractive to insects, easy to establish, robust, and grow fast enough to not be outcompeted by the corn crop. However, CB are higher in crude protein (CP) and ash content compared to corn, which could buffer a fast drop in silage pH and have negative consequences on overall silage quality. This study was designed to investigate the impact of increasing percentages of CB inclusion on the quality and aerobic stability of mixed crop corn silage. Whole crop corn (variety: Ronaldinio) was ensiled with 0% (CON), 10% (10CB), 30% (30CB), 50% (50CB; dry matter [DM] basis) Phaseolus vulgaris (variety: Meraviglia de Venezia). Per treatment, three PVC-mini silos (3.4 L) were manually filled and compacted to 250 kg DM/m3 using a pneumatic press. The mini silos were sealed by rubber lids held on by metal clamps. Each silo had a gas relief valve. The silos were stored at 16.5 ± 1.5°C and opened after 8 wk of ensiling. Silages were subsampled and analyzed for crude nutrients, pH, and fermentation acids. In addition, the DM loss (DML) was calculated. As indicator for aerobic stability, heat loss from the silages over 14 d of aerobic exposure was recorded using Thermochron iButtons (Embedded Data Systems, Lawrenceburg, KY, USA). Data were analyzed using the mixed model procedure (PROC MIXED) of SAS (version 9.4). The model included the fixed effects of treatment. Mini silo was included as random effect. The impact of increasing concentration of CB on silage quality was examined using linear and quadratic orthogonal contrasts. Significance was declared at $P<0.05$. As expected, adding CB led to a linear increase in crude protein content of the silages ($P<0.01$; CON: 6.6%, 10CB: 7.7%, 30CB: 10.2%, 50CB: 12.2% CP, DM basis). In contrast, the energy content of the silages decreased linearly ($P<0.01$; CON: 11.25, 10CB: 11.19, 30CB: 10.86, 50CB: 10.76 MJ ME/kg DM). Silage pH increased linearly when CB were added ($P<0.01$) but was below pH 4 in all treatments (CON: 3.85, 10CB: 3.86, 30CB: 3.88, 50CB: 3.90). Acetate concentrations response to increasing percentages of CB in the silages also increased linearly ($P<0.01$), while butyrate concentrations tended to linearly increase ($P=0.09$). Total heat loss (i.e., area under the curve) over 14 d of aerobic exposure was higher when CB inclusion increased (linear: $P<0.05$). In addition, the first heat loss of the silages containing CB occurred earlier (d 4 to 5) compared to the CON (d 7). In conclusion, cropping corn together with CB might have positive impact on insect biodiversity but silage quality has to be monitored carefully, as fermentation quality and aerobic stability of these polycrop silages (corn plus CB) might be negatively affected.

Degradation Kinetics of Forage Monocultures and Multispecies Swards Harvested at Two Maturity Stages

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Multispecies swards are promoted in grassland-based ruminant systems to enhance productivity and resilience under variable management and environmental conditions. Alongside species composition, maturity at harvest strongly influences forage nutritive value: less mature forage generally has higher crude protein (CP) and digestibility and lower fibre concentration. However, how forage maturity interacts with species composition to influence ruminal dry matter (DM) and CP degradation is less clear. Therefore, we evaluated the effects of forage species on the response of DM and CP degradation kinetics to harvest maturity.A field experiment established in spring 2023 included 8 forage species treatments: monocultures of perennial ryegrass (G1), timothy (G2), white clover (L1), red clover (L2), chicory (H1), and plantain (H2); a mixture of all 6 species (6Sp); and high-nitrogen (N) perennial ryegrass (G1-highN). All treatments received 100 kg N ha^{-1} annually, except G1-highN (200 kg N ha^{-1}). Treatments were replicated in three plots (n=3), except G1-highN (n=5). Plots (4 × 12.5 m) were split into early (5 harvests) and late (4 harvests) cutting subplots. Forage for rumen degradation analysis was collected at the second harvest on 29 May 2024 (early cut; 28 d regrowth) and 10 July 2024 (late cut; 56 d regrowth). Four rumen-cannulated Holstein dry cows were used for in situ incubations. Dried and milled (2 mm) forage samples were incubated in the rumen using Dacron bags for 0, 3, 6, 12, 24, 48, and 72 h, and residues were analysed for DM and CP. Degradation kinetics were modelled to estimate the soluble fraction (a), potentially degradable fraction (b), fractional degradation rate (c), potential degradability (PD=a+b) and undegradable fraction (U=100−a−b). Estimated fractions and degradation rate were analysed using linear mixed models in SAS (PROC MIXED), with forage species treatment, maturity, and their interaction as fixed effects, and plot, cow, and incubation batch as random effects.Forage species significantly affected all DM kinetic parameters ($P<0.001$), with significant forage species × maturity interactions for a, b, PD, and U ($P<0.01$). Increased forage maturity at harvest reduced PD across species ($P<0.001$), with larger declines observed in herbs (H1, 87.4 to 68.2%, early to late cut) and legumes (L1, 87.1 to 75.2%) than in grasses, including G1-highN (84.9 to 81.5%). Legume and herb monocultures exhibited higher c values (0.10 to 0.13 h^{-1}) than grass monocultures (0.07 to 0.08 h^{-1}; $P < 0.001$), indicating faster ruminal DM degradation. However, maturity did not affect c ($P=0.823$), indicating that maturity altered degradation extent and not rate. The 6Sp showed PD comparable to grasses at early cut and similar maturity-related reductions to most monocultures. For CP, forage species and maturity affected a, b, PD, and U ($P<0.001$), whereas c was unaffected ($P>0.05$). Early cut increased CP PD and reduced U. Legumes showed the highest CP PD at both cuts (L1, 92.3 to 81.9%; L2, 90.5 to 80.4%, early to late cut), while herbs exhibited larger maturity-related declines (H1, 86.1 to 62.5%; H2, 68.5 to 53.7%). Grasses showed smaller reductions (G1, 80.9 to 67.5%; G1-highN, 84.7 to 76.3%; G2, 78.6 to 73.5%). The 6Sp maintained intermediate CP PD and a smaller maturity-related increase in U than herbs.Overall, forage species and maturity altered DM and CP degradation primarily through changes in the degradable fraction. Legumes had greater ruminal protein degradability, whereas herbs were more sensitive to advancing maturity. The 6-species mixture moderated maturity-related shifts in degradability, indicating functional stability within grassland-based systems.

Red clover in grass silage-based dairy systems: A meta-analysis

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Red clover (RC; Trifolium pratense) is a high-yielding legume capable of fixing atmospheric nitrogen (N), making it an important component of crop rotations and grasslands by helping reduce reliance on fossil-based N fertilisers. Additionally, protein degradation from cutting to ensiling and later in the rumen is lower in RC than in grasses due to the activity of the enzyme polyphenol oxidase. The aim of the study was to evaluate the effects of replacing grass silage (GS) with red clover silage (RCS) on milk production and nutrient supply. This meta-analysis compiled data from published studies, including 85 silage observations from digestibility trials, 33 dietary comparisons with a total of 94 treatments from production experiments, and 16 treatments from four digesta flow studies. Digestibility trials were conducted at MTT Agrifood Research Finland, using sheep, while production and flow data were extracted from published studies on dairy cows. Data were analysed using a mixed model procedure of SAS with study as a random effect and the proportion of RCS (0 – 1 on DM basis) as the independent variable. Compared with GS, RCS had lower NDF of 370 ± 15.2 g kg^{-1} DM vs. 570 ± 10.3 g kg^{-1} DM ($P < 0.01$) and higher soluble fibre concentration, mainly pectin, resulting in faster degradation of digestible fibre. However, the higher indigestible NDF content in RCS compared to GS 109 ± 9.7 g kg^{-1} DM vs. 85 ± 6.7 g kg^{-1} DM ($P < 0.05$) generally contributed to lower total tract digestibility of NDF 620 ± 15.9 vs. 726 ± 11.1 g kg^{-1} ($P < 0.01$), while OM digestibility was not significantly different between silages 704 ± 12.6 vs. 723 ± 8.8 g kg^{-1} ($P = 0.22$). Replacing GS with RCS increased forage and total dry matter intake by 1.7 and 1.6 kg d^{-1}, respectively ($P < 0.01$). Responses to RCS were positive, particularly when fed in mixtures with GS, with milk production increasing by 2.40 kg d^{-1} with increasing replacement of GS by RCS in the diet ($P < 0.01$). However, milk protein and fat concentrations declined by 0.80 and 2.82 g kg^{-1}, respectively ($P < 0.01$) with increasing replacement of GS by RCS, likely due to dilution from increased yield when feeding RCS in the diets. Metabolisable protein intake increased by 209 g d^{-1} ($P < 0.01$); however, N utilisation efficiency decreased by 64 g kg^{-1} ($P < 0.01$) as GS was replaced by RCS in the diet, likely due to higher dietary crude protein levels, which increased N excretion and limited effective use of metabolisable amino acid (AA). Consistent with this, omasal flow of total AA increased by 706 g d^{-1} ($P = 0.03$) with increasing replacement of GS by RCS. Methane (CH_4) emission responses were small and inconsistent, with no significant difference in daily CH_4 production between low and high RCS diets by 463 vs. 475 g d^{-1} ($P = 0.13$). Overall, production of RCS offers agronomic and nutritional advantages but requires careful formulation to optimise N efficiency and milk protein synthesis in dairy systems.

A comparison of Good Agriculture Practices (GAPs) for sustainable dairy farming in South Africa with the RISE 3.0 (Response-Inducing Sustainability Evaluation) tool

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Good Agriculture Practices (GAPs) is a central framework to promote sustainable agriculture through environmental stewardship, animal welfare, social responsibility and food safety. In South Africa, the adoption of GAPs is driven by the Dairy Standard Agency (DSA), using structured audit frameworks to evaluate on-farm compliance. The real-world efficacy of DSA_GAP Sustainability audits in promoting sustainable outcomes and the alignment with other sustainability assessment tools remains largely unexplored. The RISE 3.0 is recognised as an indicator-based method for benchmarking sustainability on dairy farms. DSA_GAP audits mainly assess compliance with structured, risk-based standards and regulatory requirements, while RISE 3.0 audits evaluate broader sustainability performance through a indicators-based, outcome-focused assessment across environmental, social, and economic aspects. This study aimed to assess the effectiveness of GAPs in achieving sustainability goals through a comparative analysis of the DSA_GAP Sustainability assessment audit and RISE 3.0 evaluation reports. From 512 dairy farms evaluated in the GAP analyses representing TMR, pasture based and hybrid farms in four regions, 24 farms with verified records and sufficient RISE 3.0 report outcomes across environmental, social responsibility, product compliance, and risk management topics, were considered. Spearman correlations were estimated across 13 comparative theme combinations based on the GAP and RISE sub-theme scores and correlation strengths interpreted using the Asuero et al. (2006) scale. Across of themes, correlations were weak p=0.405 (environment – material) to moderate p=0.466 (social responsibility). In the Social Responsibility theme, a weak moderate correlation (0.348) was found for Animal Husbandry, while no significant correlation was found with Animal Welfare. For the Risk/Economic theme, both Risk Management and Documentation and Information showed weak and non-significant monotonic associations with DSA_GAP Sustainability scores ($\rho < 0.31$; $P > 0.05$). The results demonstrate selective alignment between DSA_GAP Sustainability and RISE 3.0 indicators, primarily within social responsibility and material-use dimensions, while most environmental and risk-related sub-themes show weak or inconsistent monotonic relationships. Although most sub-themes did not reach statistical significance, the overall positive directional trend across themes suggests conceptual alignment between compliance-based GAP implementation and broader sustainability performance. These findings support international evidence that structured management practices contribute positively to environmental and social sustainability outcomes and confirm that certification-based and performance-based frameworks capture overlapping but non-identical dimensions of sustainability. In practice, assessment approaches that combine compliance-based GAP audits with performance-based environ mental metrics could offer a more comprehensive view of farm-level sustainability.

AgroServ: an integrated multi-RI platform supporting agroecological transition in cattle production

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Agriculture today faces a complex set of pressures, with agricultural lands threatened in two aspects: the impact of climate change and the environmental and social consequences of current agricultural practices. To address these challenges, AgroServ, funded by the EU's Horizon Europe program (grant No. 101058020), was launched as a transdisciplinary initiative bringing together researchers, industry, policymakers, and farmers. With 73 partners in over 20 countries, AgroServ provides 143 research services through 12 research infrastructures (RIs). These RIs cover molecular to ecosystem-level sciences, including SmartCow, a leading RI in livestock research, that provides advanced access to diverse cattle types, husbandry systems and cutting-edge animal science technologies to strengthen AgroServ's focus on sustainable animal production. Since 2022, AgroServ has enabled innovation through excellence-based selection of projects combining multiples RIs. SmartCow supports 4 projects, collaborating with other RIs to bridge animal sciences with AgroServ's broader agroecological services, fostering interdisciplinarity solutions. These projects focus on the use of forage legumes, vegetable by-products and feed additives to reduce enteric methane emissions, while maintaining animal performances and quality of animal products. Four Transnational/Virtual Access (TA/VA) calls have been completed (two more planned in 2026), attracting a balanced mix of early-career (57.9%) and established researchers (42.1%). Selected projects (49 to date), represent diverse institutions, including universities (64%), applied research centres (26%), industry (9%) and government (2%). The geographical reach of the proposals was also broad, with global participation, including several low- and mid-income countries. AgroServ's legacy will extend beyond 2027, ensuring continued support for agroecology through enduring networks, open-access services, and cross-border knowledge exchange. By bridging science, policy, and practice, AgroServ empowers resilient, sustainable food systems worldwide.

Exploration of resilience metrics in grazing dairy cows

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Grazing dairy systems require animals with enhanced adaptive capacity to fluctuations in weather and nutrition, making resilient cows highly desirable. Resilience is the ability to be minimally affected by or to rapidly return to the unperturbed state. Time-series data, such as daily milk yield, are required to quantify and rank animals within a herd. Most resilience indicators were developed in confined systems, and their applicability to pasture-based systems remains uncertain. LnVar, calculated from daily milk yield without explicitly identifying macro-perturbation events (MP), may provide a practical resilience metric under grazing. However, due to high daily variability, it is unclear whether LnVar reflects individual resilience to MP. This study evaluated whether lactation-level and stage-specific LnVar reflect individual resilience and cow rankings within the herd, compared to event-based traits rankings during MP in a commercial dairy grazing system in Uruguay. Eighty-three autumn-calving multiparous Holstein cows (mean milk yield: 28.8 ± 5.0 kg/day; mean lactation length: 298 ± 27 DIM), monitored throughout their 2024 lactation, were analyzed. LnVar was calculated as the natural logarithm of the variance of daily milk yield residuals. Predicted milk yield was estimated using a 5th-order polynomial quantile regression (0.5 quantile). LnVar was computed for the entire lactation and for three stages: 21–100, 100–200, and 200–300 DIM. MP were defined as periods of ≥5 consecutive days with a ≥5% decrease in herd milk production relative to potential production. Three MP were identified (MP1–MP3). MP1 (April 22–July 1) was likely associated with rainfall and declining temperatures, while MP2 (October 7–November 18) and MP3 (November 25–December 16) were linked to feeding changes. The herd averaged 45, 213, and 262 DIM at the onset of MP1, MP2, and MP3, respectively. For each MP, the milk loss velocity (Vpert), recovery velocity (Vrecov), and total milk loss (%Loss) were estimated. Cows were ranked within the herd for each trait. Spearman correlations were used to evaluate associations among rankings. Repeatability of each trait was estimated using the intraclass correlation coefficient (ICC) derived from a linear mixed model with individual as a random effect. Concordance of rankings across lactation stages and MP was assessed using Kendall's coefficient of concordance (W). Correlations showed positive associations between overall LnVar and stage-specific LnVar ($r > 0.63$; $p<0.0001$), with the strongest correlation observed for 21–100 DIM ($r = 0.78$; $p<0.0001$). Both overall LnVar and 21–100 DIM LnVar were negatively correlated with %Loss during MP1 ($r = –0.58$ and $–0.49$, respectively; $p<0.0001$). Repeatability was low for all traits (ICC<0.18). However, Kendall's W indicated moderate agreement for LnVar rankings across lactation stages ($W = 0.57$; $p<0.01$), while concordance for Vpert across MP was weaker and non- significant ($W = 0.42$; $p = 0.067$). Vrecov and %Loss showed low and non-significant agreement ($W <0.40$; $p>0.10$). LnVar consistently reflected individual rankings, with early lactation influencing overall LnVar due to higher milk yield and increased susceptibility to perturbations. The association between LnVar and milk loss during MP1 suggests that perturbations occurring in early lactation contribute more strongly to LnVar than events occurring later or with lower impact. Despite moderate consistency in rankings, the low repeatability indicates that within-individual variation exceeded between-individual variation. These findings suggest further research is needed to validate LnVar as a robust resilience indicator for identifying superior animals under challenging conditions.

Resilience indicator and productive performance in a commercial grazing dairy farm

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Dairy cows under grazing conditions are particularly required to be resilient to environmental challenges. This study explored the relationship between the resilience indicator LnVar across the entire lactation and animal performance in a commercial grazing dairy farm in Uruguay. Eighty-three autumn-calving multiparous Holstein cows (mean milk yield: 28.8 ± 5.0 kg/d; mean lactation length: 298 ± 27 DIM; parity: 3.2 ± 1.2) from a commercial dairy farm located in the main dairy basin of Uruguay (temperate climate) were monitored throughout their 2024 lactation. During the prepartum period, cows were fed a corn silage–based TMR, whereas during lactation they grazed alfalfa and oat pastures and were supplemented with a corn silage–based TMR at a feedpad (5–10 kg DM/d). Milk yield was recorded daily, and body condition score (BCS, 1–5 scale) and body weight were assessed at −30, at parturition, and at 21, 100, and 200 ± 15 DIM. Resilience was quantified as LnVar, calculated as the natural logarithm of the variance of daily individual milk yield residuals. Predicted milk yield was estimated using a fifth-order polynomial quantile regression (0.5 quantile). Higher LnVar values indicate lower resilience. Cows were ranked according to LnVar values, and animals with the highest and lowest resilience were selected (High-Res, n = 8; Low-Res, n = 8). Data were analyzed using mixed models including parity and DIM as covariates. High-Res and Low-Res cows differed significantly in LnVar (2.32 vs 3.34 ± 0.04, mean ± SEM; $P < 0.001$). High-Res cows showed lower mean milk yield (26.5 vs 30.7 ± 1.2 kg/d, $P = 0.0306$), lower peak milk yield (31.9 vs 38.6 ± 1.56 kg/d, $P = 0.032$), and lower accumulated milk yield (7,700 vs 8,973 ± 307 kg, $P = 0.015$). However, body condition score at 21 DIM was greater in High-Res cows compared with Low-Res cows (3.3 vs 2.7 ± 0.12, $P = 0.01$). These results suggest that cows classified as highly resilient tended to have lower milk production but better body condition during early lactation. However, it remains unclear whether this relationship reflects a cause or a consequence of resilience, and further research is needed to clarify this association. The greater body condition may indicate a more favorable nutritional status, which could potentially translate into improved reproductive efficiency.

Effect of incorporating Bromus valdivianus Phil. on the nutritive value of a mixed pasture during the spring–summer season in southern Chile

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Nutritive value varies with climatic conditions and plant phenology, making the inclusion of nutritionally stable forage species essential for sustaining milk production. Consequently, Bromus valdivianus Phil., a native species from southern Chile, shows strong summer performance and a high nutritive value. The objective of this study was to evaluate the nutrient concentration of a polyphytic pasture (Pol) composed by Lolium perenne L., Trifolium repens L., and Bromus valdivianus Phil., compared to a mixed pasture (Mx; Lolium perenne L. and Trifolium repens L) during the spring–summer period in southern Chile. Three sectors were used, each divided into two pasture types: one Mx and one Pol. Sampling was conducted weekly using a 0.25 m² quadrat thrown randomly three times, cutting at 5 cm above ground level, with a total of 554 samples collected across both seasons. Regarding climatic conditions during the study, in spring 2024 temperatures ranged between 5–25 °C with constant rainfall of 5–10 mm, while in summer temperatures ranged between 15–35 °C and precipitation was concentrated at the end of the season, reaching 70 mm. Nutritive value was estimated using near-infrared reflectance spectroscopy (NIRS). The results showed that nutritive value concentrations (CP %, ME Mcal kg^{-1} DM, NDF %, ADF %, and NSC Mcal kg^{-1} DM) did not differ between pasture types during spring. In summer, polyphytic pasture showed higher crude protein (CP) concentrations (+0.85 percentage units) than that of MX (18.9 vs. 18.05% for Pol and Mx, respectively). Neutral detergent fiber (NDF) was also higher in Pol (59.48%) than in Mx (57.92%; +1.56 percentage units), while acid detergent fiber (ADF) averaged 30.31% in Pol and 29.10% in Mx (+1.21 percentage units). In contrast, metabolizable energy (ME) did not differ between pasture types, averaging 2.62 and 2.56 Mcal kg^{-1} DM for Mx and Pol, respectively. In terms of accumulated nutrients (kg ha^{-1}), the Pol pasture showed significantly higher values than Mx across both seasons for all variables. Overall, the polyphytic pasture outperformed the mixed pasture during summer in both nutrient concentration and accumulation (CP, NDF, and ADF), supporting its suitability as a forage option for this season in southern Chile.Project financed by FONDECYT 1240451.
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Polyphenol oxidase activity, phenolic concentration and utilisable crude protein in red clover (Trifolium pratense L.) accessions

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Red clover (RC) is an important forage legume in northern Europe due to its high protein content and ability to fix N from the air through symbiosis with soil bacteria. Further, RC has high polyphenol oxidase (PPO) activity and a high concentration of phenolic substrates. Upon activation, PPO catalyses the oxidation of phenolic compounds, which subsequently interact with plant proteins to form complexes that are less susceptible to ruminal degradation. If effectively utilised, RC has strong potential as a future sustainable protein feed resource. In this study, we made use of a unique RC accession material developed within the Grogrund project "Genomic selection of red clover". The RC accessions represented synthetic cultivars, landraces and commercial varieties from the Nordic countries. We examined whether RC type and ploidy were associated with PPO activity, phenolic compound concentration, and in vitro-determined metabolisable protein (utilisable crude protein, uCP) and whether traits were interrelated. Forty-four accessions were evaluated (20 diploid and 20 tetraploid), including four control varieties (two diploids and two tetraploids). The analysed accessions consisted of synthetic, commercial and landrace varieties. Herbage was harvested at the second cut in 2023, wilted, chopped, and ensiled with formic acid-based additives in plastic bags using a vacuum machine to establish an oxygen-depleted environment. Phenolic concentration and PPO activity were measured in fresh RC leaf material. Utilisable crude protein at 16 h rumen retention time (uCP16) was estimated from the ensiled material using a fully automated gas in vitro system. Silages were also analysed at Dairy One (Ithaca, NY, USA) for Cornell protein fractions. Silages were well preserved as indicated by adequate lactic acid content (81.2 ± 13.77 g/kg DM), a negligible amount of butyric acid (0.5 ± 0.04 g/kg DM) and low ammonia-N (28.1 ± 5.41 g/kg total N). Diploid accessions had higher uCP values than tetraploids (196 ± 1.60 vs 184 ± 1.70 g/kg DM; $P < 0.01$). Compared with commercial types (184 ± 2.22 g/kg DM), landraces (195 ± 3.07 g/kg DM; $P < 0.01$) and synthetic cultivars (192 ± 1.04 g/kg DM; $P < 0.01$) showed higher uCP values. Crude protein also significantly influenced uCP ($R_2 = 0.73$; RMSE = 5.78; $P < 0.01$). Similarly, several Cornell protein fractions were significant predictors of uCP, including soluble protein ($R_2 = 0.32$; RMSE = 8.54; $P < 0.01$), insoluble true protein ($R_2 = 0.29$; RMSE = 8.70; $P < 0.05$), and indigestible protein ($R_2 = 0.30$; RMSE = 8.65; $P < 0.01$). Phenolic concentrations varied among RC types, with higher values in commercial types (28.4 ± 3.64 mg g^{-1} DM) than in landraces (23.8 ± 3.90 mg g^{-1} DM; $P < 0.05$) and synthetic populations (24.4 ± 3.59 mg g^{-1} DM; $P < 0.01$). Similarly, active PPO activity (Δ min^{-1} mg^{-1} FM) differs among RC types ($P < 0.01$), with the highest values observed in landraces (292 ± 78.3), followed by commercial cultivars (180 ± 69.3) and synthetic cultivars (143 ± 67.5). The differences observed among the RC accessions suggest genetic diversity in protein utilisation traits. Variation in RC type and ploidy levels across these traits can be strategically leveraged to enhance breeding programmes aimed at improving protein utilisation efficiency.

Winter grazing or bale haylage fed indoors for pregnant heifers: growth performance and synthesis of welfare and behavioral indicators

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Climate change is leading to milder winters and a persistence of grass growth later into autumn and even winter in the most oceanic regions. Since 2021, trials have been conducted at the Trévarez experimental station (western France) to provide reference data on the nutritional value of autumn/winter grass and its utilization by dairy heifers in an oceanic climate. The objective of this work was to study the potential use of winter grass by dairy heifers and to compare the performance of these animals with that of heifers kept indoors and fed bale silage. These results are complemented by animal welfare and behavioral data to evaluate the impact of winter grazing on animals under weather conditions considered potentially less favorable than in spring. A trial was therefore carried out over three winters from 2021 to 2023. Each year, twelve pregnant Holstein heifers raised under conventional farming conditions were divided into two homogeneous groups (IND group and GRAZ group) and paired accordingly. The trial began between November 4 and 14 depending on the year and ended between February 6 and 9, for a total duration of 85 to 97 days. Heifers in the IND group spent the winter indoors on a straw-bedded area and were fed wrapped round bales (bale silage) without any concentrate supplementation. Heifers in the GRAZ group spent the winter at pasture, without any supplementary forage or concentrate. Each winter, the grazing heifers used between 9 and 12 paddocks averaging 1.3 ha (0.75 to 2.59 ha), with an average residence time of 9 days per paddock (2 to 17 days) and an instantaneous stocking rate of 3 livestock units per hectare. During the trial, rainfall ranged from 186 mm to 528 mm depending on the winter, with an average temperature of 7 °C and between 7 and 20 days of frost (down to −7 °C). The crude protein content of winter-grazed grass was high (20–22% CP in DM) and higher than that of bale silage. Average daily gains of the two groups were not significantly different and met the target levels over the three winters (826 to 1094 g/day for the IND group and 907 to 1005 g/day for the GRAZ group). The indicators selected to assess animal welfare (body condition score, lameness, and injuries) were derived from the Welfare Quality Protocol (2009) and the Boviwell tool. The welfare evaluation was only performed during one winter (2023/24). The welfare indicators did not differentiate between the two groups. However, a difference was observed regarding cleanliness: animals became dirty indoors from December onwards, whereas they remained clean at pasture throughout the trial. Twice as many social behaviors were observed indoors compared with grazing, mainly agonistic behaviors. At the end of the trial, no marked difference was observed between the two groups regarding the human–animal relationship. The nutritional value of winter grass and its utilization by heifers over the three years indicate that it is a forage of real interest. Grazing carried out under the conditions described did not appear to cause particular difficulties for the pastures. Under the winter conditions of 2023–2024, winter grazing of pregnant heifers did not reduce animal welfare. The quality of social interactions also appeared less negative at pasture, with less competition between animals.

Chemical composition, in vitro digestibility, and methane production of selected fodder trees in late summer

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Climate change increasingly exposes livestock systems to recurrent summer droughts, reducing pasture productivity and the availability of high-quality forage. In this context, fodder trees gain renewed interest as resilient resources thanks to their deep roots and ability to supply biomass when herbaceous vegetation declines. This study evaluated the chemical composition, in vitro digestibility, methane (CH_4) production potential, and palatability of leaves from five tree species (Alnus cordata, Fraxinus ornus, Morus alba, Salix caprea, and Tilia cordata). Leaves were collected in August 2024 from forage hedgerows established on grassland plots within the AgroForageTree project in Western Switzerland. A short-term palatability test was conducted on six castrated male sheep (license 2024-14-FR) to assess intake of the leaf species compared with a control hay (grass-rich grassland, 1st cut, full heading). The in vitro organic matter digestibility (IVOMD) and CH_4 production were quantified using the Hohenheim Gas Test. Five diets consisting of 80% control hay and 20% freeze-dried leaves (DM basis), plus a 100% hay control, were incubated in three runs using rumen fluid from two rumen-cannulated dry cows (license ZH113/18), with three analytical replicates per diet. Condensed tannins were analysed by the HCl-butanol method, and diet chemical composition by Weender analysis. After 24h incubation, pH, ammonia, and volatile fatty acids (VFA) were measured in the liquid phase. Total gas production over 24 h was recorded, and CH_4 and CO_2 concentrations were determined by gas chromatography. Data were averaged by replicate and analysed in R using linear mixed-effects models with tree species as fixed and run as random effect. Salix caprea was the most palatable species (palatability index 106%), while Alnus cordata was the least consumed (palatability index 49%). Mixed-diet IVOMD ranged from 57 to 66%, with the highest digestibility ($P < 0.01$) for Morus alba (66±0.9%), similar to the control hay (65±0.9%). The lowest IVOMD ($P < 0.01$) occurred with Alnus cordata (57±0.9%), while the remaining species showed intermediate values. Total VFA were highest for Morus alba (59±2.2 mM) and Tilia cordata (58±2.2 mM), and lowest for Alnus cordata (52±2.2 mM). The Morus alba diet also yielded the highest ammonia concentration (9.9±0.5 mmol/L; $P < 0.01$), consistent with the elevated crude protein content of M. alba leaves (198±8.8 g/kg DM). The lowest CH_4/CO_2 ratio was observed with Salix caprea (143±6.7 ml/l), likely due to its higher condensed tannin content ($P < 0.01$), known to inhibit methanogenesis. In contrast, Fraxinus ornus, Morus alba, and the control hay produced the highest CH_4/CO_2 ratios (151±6.7 ml/l; $P < 0.01$). In summary, Morus alba showed the most promising nutritive characteristics, while Salix caprea displayed the strongest potential for reducing CH_4 emissions in vitro. Further research, including in vivo trials at farm scale, is needed to evaluate biomass production, intake, and the integration of fodder trees into grazing systems.

Agolin and Polygain Supplementation Reduces Methane Production Without Affecting Blood Metabolites in Grazing Sheep Under Commercial Farm Conditions

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Enteric methane from ruminant livestock contributes substantially to agricultural greenhouse gas emissions and represents an energy loss accounting for up to 12% of dietary intake in sheep. Feed additives such as plant essential oils and polyphenol extracts have shown potential to reduce methane production while maintaining productivity in ruminants. However, most studies have been conducted under controlled conditions, and evidence from commercial production systems remains limited. This study tested the hypothesis that supplementation with Agolin (an essential oil blend) and Polygain (a sugarcane polyphenol extract), individually or combined, would reduce methane emissions in grazing lambs without negatively affecting growth performance or animal health. A total of 200 crossbred lambs (≈3 months old) were allocated to four treatment groups (n = 50 per group): control, Agolin (0.0002% of the body weight per day), Polygain (0.25% of dry matter intake (DMI) per day), and a combination of Agolin and Polygain in half doses each. Animals grazed pasture in separate paddocks on a commercial farm in Northern Victoria and received daily pellets blended with the assigned supplements for 80 days. Methane emissions were measured rotating groups through a GreenFeed emission monitoring unit during multiple measurement periods. Body weight was recorded every three weeks. Blood metabolites were analysed to assess metabolic and health status before and after supplementation. Data were analysed using mixed-model statistical procedures in GenStat 23rd edition with treatment as a fixed effect and individual animal as a random factor. Initial body weights were similar across groups (~37 kg), confirming baseline uniformity. Supplementation did not affect body weight gain or average daily gain throughout the study. Methane production (g/day) was significantly lower in Agolin (35), Polygain (36.9) and combination (34) groups compared to the control group (38.5) ($P < 0.001$). Methane intensity was numerically reduced by 9.78% with the combined additives and by 5.96% with Polygain alone ($P < 0.10$). Most blood biochemical indicators, including urea, albumin, non-esterified fatty acids, liver enzymes, and creatinine, were not affected by the treatments, suggesting no negative impact on animal health and physiology. Overall, Agolin and Polygain supplementation did not compromise growth performance or key metabolic health indicators in lambs. The significant reduction in methane production, particularly when the additives were combined, indicates potential additive effects in sheep under commercial grazing conditions. These findings support the hypothesis that feed additives can reduce methane emissions without reducing productivity. Further study with longer monitoring periods is needed to confirm the magnitude of the mitigation effect and evaluate the economic feasibility of adoption in commercial farms.

Modifying aspects of ruminal fermentation to mitigate enteric methane generation in goats through the use of saponin- and tannin- rich plant sources - A meta-analysis.

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The present meta-analysis attempts to integrate data from various studies in order to increase statistical power, identify the research gaps, resolve conflicts, and provide up-to-date, comprehensive information on the use of saponin- and tannin-rich plant sources to improve rumen fermentation products as well as to mitigate enteric methane (CH4) generation in small ruminants, particularly, goats. Thirty-two (32) studies retrieved from Google Scholar, Web of Science, Scopus, and PubMed and extracted data on Dry matter intake (DMI), fermentation efficiency (FE), rumen- pH, -ammonium- nitrogen (NH3-N), total volatile fatty acid (TVFAs), -bacteria population, -protozoa population, and CH4 from these studies were subjected to random-effects model in OpenMEE statistical software for meta-analysis. The results were presented as standardized mean difference (SMD) at a 95% confidence interval (CI). Meta-analysis results revealed a significant increase in propionate (SMD= 0.71; CI= 0.21, 1.21) and a reduction in the ratio of acetate to propionate (SMD= -0.98; CI= -1.57, -0.38). Furthermore, pooled results revealed a significant reduction in NH3-N (SMD= -1.34; CI= -1.86, -0.83), protozoa population (SMD= -1.08; CI= -1.95, -0.22) and CH4 (SMD= -0.84; CI= -1.16, -0.51). Subgroup analysis showed that higher inclusion levels (>30%) had increased acetate, the ratio of acetate to propionate, and bacterial populations. However, NH3-N and protozoa populations were reduced by higher inclusion levels (>30%). Similarly, feeding duration, sex, and phytochemical compounds were predictors for propionate, butyrate, NH3-N, bacteria, and protozoa population in goats supplemented with saponin- and tannin-rich plant sources. Meta-regression analysis using a moderator revealed negative relationships between NH3-N, bacterial population, protozoa population and sex. Similarly, feeding duration was linearly related to bacteria population, while inclusion level was linearly related TVFAs, NH3-N, bacteria population, and enteric CH4. In conclusion, supplementing saponin- and tannin-rich plant sources improved rumen fermentation parameters and successfully reduced protozoa populations and enteric CH4 generation, with no adverse effects on DMI. Key words: phytochemical compounds, fermentation parameters, rumen characteristics, ruminants, rumen microbes

Effects of individual and combined feed additives on ruminal in vitro fermentation characteristics.

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To achieve sustainable agricultural and food security, livestock production efficiency needs to improve. Enteric methane (CH4) from ruminants represents both a dietary energy loss and a major greenhouse gas emission. Whilst many feed additives reduce methane in vitro, effects are inconsistent and may disrupt fermentation characteristics. Combining additives with differing modes of action may stabilise results, benefit other fermentation characteristics, and reduce microbial adaption for long-term use. This study assessed seven feed additives individually and in combination at two doses (full and half doses that were tested individually). Additives included seaweeds (Ulva sp. [UV1] and Solieria chordalis [SC1]), essential oils (capsicum [CAP], oregano [ORE] and Cinnagenol [CNG]; cinnamaldehyde:eugenol 3:1) and commercial or near-market additives (Golf [GLF] and Feed Additive 1 [FA1]). Batch fermentations were conducted 3 times in 100 mL serum jars using a grass silage (perennial ryegrass) substrate and filtered rumen fluid collected from abattoir from 5 animals, buffered with Van Soest (1:1 ratio). Additives were analysed in triplicate alongside blank and control incubations for 24h at 39°C. Measurements included pH, gas production, ammonia-N, digestibility, methane production and yield, and volatile fatty acid (VFA) profiles. Data were analysed in RStudio using ANOVA or Welch's ANOVA with Tukey or Dunn post-hoc tests, when significant ($P<0.05$). Individually, FA1 was the only additive to significantly reduce CH4 production and yield (-53.6% and -54.7%, respectively) relative to the control. FA1 also significantly reduced total gas production (-18.3%) and increased pH (3.6%), whilst non-significantly increasing digestibility (5.7%), ammonia-N (13.8%), Acetate:Propionate (A:P) ratio (11.6%) and total VFA (8.1%). No additive significantly altered ammonia-N, digestibility or VFA production. Additive combinations using FA1 as a base maintained significant CH4 reductions. The most consistent responses occurred with FA1+CNG and FA1+ORE. FA1+CNG reduced CH4 production by 53.8% (full dose) and 50.2% (half dose), whilst FA1+ORE reduced CH4 production by 52.2% (full dose) and 52.4% (half dose). Dry matter digestibility increased numerically across all treatments, with the greatest increases observed for FA1+CNG and FA1+ORE full doses (24.9% and 27.8%). Methane yield declined significantly for all combinations; FA1+CNG and FA1+ORE full doses reduced yield from 6.43 mL/g digested organic matter to 2.33 and 2.32 mL/g, equivalent to reductions of 60.7% and 62.2%, respectively. Only these two combinations reduced the A:P ratio at both doses, however not-significantly. Total gas production decreased across treatments, significantly for FA1+ORE at both doses. Total VFA was numerically decreased by all treatments except for the FA1+GLF half dose and FA1+CAP full dose. The pH was significantly increased in all treatments, whereas ammonia-N and individual VFA fractions were unaffected by all treatments. Overall, additive combinations sustained CH4 mitigation without detrimental effects on key fermentation parameters. Although statistical significance was not observed for digestibility, ammonia-N, or A:P ratio, numerical improvements indicated potential interactions were present, particularly for FA1+CNG and FA1+ORE. The stated combinations highlighted improvements beyond that of stacking the results of the additives individually. These findings suggest combining feed additives, with differing modes of action, may enhance methane mitigation strategies whilst supporting rumen fermentation and therefore warrant further in vivo evaluation for application within the livestock sector.

Assessment of some Mediterranean shrubs by in vitro studies: total gas production and CH4, CO2, H2, O2, N2 profiles

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In Mediterranean countries, shrub species often represent the main available feed resource for grazing ruminants, particularly when grass is scarce or inexistent. Extensive livestock systems, plays a crucial socio-economic role and is frequently one of the few viable economic activities. Woody species are generally characterized by moderate crude protein (CP) content and high concentrations of phenolic compounds; however, these values can vary considerably among species and according to phenological stage. The low nutritional value of shrubs is largely affected by their lignified fraction, phenols and other plant secondary compounds. Although plant secondary metabolites are commonly regarded as antinutritional factors in ruminants, phenolic compounds are known to inhibit ruminal biohydrogenation and may contribute to methane production mitigation. The objective of this work, carried out as part of the LIFE Scrubsnet project, was to evaluate the effect of different shrub species on ruminal fermentation with particular emphasis on methane production. Twig samples (mainly leaves) from shrubs collected in Spain, Portugal and Italy were characterized for their chemical composition and assessed for in vitro total gas production and gas profile (CH4, CO2, H2, O2 and N2). The shrub species evaluated were Arbutus unedo (AU), Quercus ilex (QI) and Morus alba (MA) and they were compared with corn silage (CS) as a control. Two fermentation runs were performed. Samples (440 ± 0.2 mg) were incubated in triplicate with 40 mL of medium and 20 mL of rumen fluid. The average NDF content (% DM) was 20.94, 50.64, 35.11 and 41.79 for MA, QI, AU and CS respectively. Crude protein content (% DM) was 19.00, 8.92, 8.15 and 7.78 while phenols (% DM) were 2.31, 10.14, 9.78 and 0.00 for MA, QI, AU and CS respectively. Total cumulative gas production (GP) as well as CH4, CO2, H2, O2 and N2 were measured at 24 hr of incubation and expressed as mL/g DM incubated. Data were analysed using a GLM model including forage species as a fixed effects. The relationship between gas production and the chemical composition of the studied species was analyzed using linear regression models. The total gas production was significantly higher ($P < 0.001$) in MA and CS compared with AU and QI. A similar trend was observed for methane emission, with MA and CS showing approximately twofold higher CH4 emissions ($P < 0.001$) than AU and QI. The CO2 production was higher ($P < 0.01$) in MA compared with the other treatments. The NDF content affected negatively the total gas production ($r_2 = -0.39$; $P < 0.01$) and CH4 ($r_2 = -0.30$; $P < 0.01$). The phenols content affected negatively the total gas production ($r_2 = -0.50$; $P < 0.01$) and CH4 production ($r_2 = -0.27$; $P < 0.01$). No significant differences were detected among the forage species for N2, H2 and O2 production. These results confirm that Mediterranean shrubs species can significantly modulate ruminal fermentation patterns, potentially contributing to improved environmental sustainability of extensive livestock systems through reduced methane emissions.

Dose-Dependent Modulation of Methane Production, Rumen Fermentation, Digestibility and Rumen Microbial Community by L-Carvone: An in vitro Study

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There is growing interest in natural feed additives to mitigate ruminal methane emissions. This study evaluated L-carvone (LC), a monoterpene compound from essential oils, for its dose-dependent effects on rumen fermentation, in vitro dry matter digestibility (IVDMD), methane production, and microbial composition. Three treatments were tested: a basal diet (total mixed ration; TMR + 0 µL/L LC), LC250 (TMR + 250 µL/L LC), and LC500 (TMR + 500 µL/L LC), using a 59:41 concentrate-to-forage substrate. Fermentation parameters were analysed using the Gas Endeavour system and 16 S rRNA gene sequencing was applied to investigate the ruminal microbiota profile. Data were evaluated via linear mixed models with treatment as a fixed factor and experimental runs as a random factor. Pairwise comparisons were performed using Bonferroni correction, with P value below 0.05 considered significant. Total gas and methane production decreased progressively with increasing LC inclusion, with significant reductions observed only at LC500 ($P < 0.001$). Similarly, LC500 reduced methane production by 28% ($P < 0.001$) and decreased IVDMD by approximately 15%, while LC250 showed a numerical but non-significant reduction in methane production without affecting IVDMD. Total volatile fatty acid (VFA) concentration was reduced in LC500 ($P < 0.01$) as compared to TMR and LC250, mainly due to lower propionate, iso-butyrate, and iso-valerate concentrations, while the proportion of butyrate increased from the TMR to LC250 and was highest at LC500 ($P < 0.05$). Ammonia-N concentration also decreased in LC500 ($P = 0.040$). Alpha diversity was highest in LC250, while beta diversity indicated significant microbiota compositional divergence among treatments ($P < 0.05$). Increasing LC supplementation reduced Bacteroidota abundance such as Prevotella genus, while increased Firmicutes, particularly butyrate-producing genera such as Butyrivibrio, Pseudobutyrivibrio, and Ruminococcus. These results suggest that LC can modulate rumen fermentation by altering VFA production and reducing methane emissions through shifts in the rumen microbiota. Optimizing the inclusion rate is therefore essential to achieve methane mitigation without impairing feed digestion.

In Vitro Assessment of Eucalyptus and Ginger Essential Oils on Methane Production in Rumen Fermentation

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The global demand for sustainable livestock farming has intensified research into strategies aimed at reducing the environmental footprint of ruminant production. Methane (CH_4), a by-product of rumen fermentation, can represent an energy loss of up to 12% for the animal and contributes to global warming due to its high greenhouse potential. Essential oils have been proposed as natural feed additives for reducing CH_4 emissions through modulation of rumen fermentation, particularly via their effects on ruminal protozoa and methanogenic bacteria. Ginger essential oil (GEO) contains bioactive secondary metabolites, that alter protozoan membrane permeability and inhibit glycolytic enzyme activity. Previous studies reported that GEO can reduce CH_4 by 16–21%, though effects on total gas, the acetate: propionate ratio and ammonia (NH_3) have been inconsistent across the studies. Eucalyptus essential oil (EEO), rich in cineole and eucalyptol, has shown stronger anti-methanogenic and antiprotozoal activity, with protozoa reductions of up to 64.5% and dose-dependent CH_4 inhibition reported in vitro, although high in vivo dosages may reduce feed palatability. We hypothesized that both oils would reduce in vitro CH_4 production compared to CTR, with EEO showing greater inhibition based on its reported anti-methanogenic and antiprotozoal properties in the literature. Therefore, this study investigates the impact of GEO and EEO on CH_4 emission and NH_3 concentration using the Gas Endeavour System® (GES; Bioprocess Control, Lund, Sweden), an in vitro rumen fermentation system. The experiment was conducted over 3 independent fermentation trials, each performed on a separate day to account for daily variability. Each fermentation included two treatment conditions (GEO, EEO) and control (CTR; TMR without additives). Both oils were tested at a single concentration of 22.5 mg/L of fermentation medium, derived from in vivo recommendations (40–50 mg/head/day) and scaled using a conversion formula accounting for rumen volume (100 L) and reactor capacity (0.5 L), with a 5-fold adjustment factor to account for the inherent differences between in vitro and in vivo conditions. CH_4 production was measured at 8h, 12h and 24h. Fermentation liquid phase samples were collected after 24h and analysed for pH and NH_3. Data were analysed using one-way ANOVA and treatment means were compared with CTR using Dunnett's test. At 24h, both EO significantly reduced CH_4 production compared to CTR (CTR: 313.7 ± 52.4 ml; GEO: 238.0 ± 50.2 ml, −24.1%; EEO: 81.8 ± 6.7 ml, −73.9%, $p<0.05$). At earlier time points, only EEO showed statistically significant CH_4 inhibition ($p<0.05$), while GEO did not differ significantly from CTR at 8h or 12h. NH_3 concentration was not significantly affected by either treatment ($p>0.05$). Overall, EEO demonstrated a stronger and more consistent in vitro anti-methanogenic effect than GEO at the tested inclusion level, with a CH_4 reduction nearly three times greater at 24h. The GEO reduction (−24.1%) is consistent with the 16–21% range previously reported in the literature, while the EEO result (−73.9%) exceeds prior estimates and aligns with its known antiprotozoal and antibacterial mechanisms of action. These findings support the potential use of EEO as a natural CH_4 mitigation strategy. Further research is required to assess its impact on broader rumen fermentation dynamics, including VFA profile and digestibility, and to confirm efficacy under in vivo conditions.

In vitro screening of potential synergistic combinations of garlic, cinnamon and oregano to decrease rumen methanogenesis

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Reducing methane (CH4) emissions while maintaining or even increasing rumen fermentation efficiency represent a challenge in ruminant production. This study aimed to evaluated the effects of combining up to three natural feed additives including garlic extract (G), and essential oils from cinnamon (C) and oregano (O) on rumen CH_4 emissions and fermentation patterns. Two in vitro experiments were conducted using batch cultures inoculated with rumen fluid from 4 cows and a 60:40 forage to concentrate diet. Experiment 1 was a dose-response trial in which 4 doses of garlic extract (up to 0.12 g/L), cinnamon EO and oregano EO (up to 2 g/L) were incubated for 24h to determine the optimal dose for the next experiment. Experiment 2 evaluated 11 treatments, including the control, individual additives (100%G, 100%C and 100%O), binary (50G-50C, 50G-50O and 50C-50O) and ternary combinations (33G-33C-33O, 75G-12C-12O, 12G-75C-12O and 12G-12C-75O). Treatments were incubated during 5 days with daily media renewal. Rumen fermentation parameters, gas and CH4 production were measured at 24h and day 5. The effects of additive combinations were further assessed using ANOVA and Bliss independence analysis to compare expected versus observed responses and to identify additive, synergistic or antagonistic effects among feed additives. In Experiment 1, garlic extract demonstrated a strong antimethanogenic potential, reducing CH_4 emissions by up to 83% and ammonia-N levels by up to 24%, without significantly affecting total VFA concentrations. Additionally, it shifted from acetate towards propionate production. High doses of Cinnamon and oregano (100–200 mg/g DM) significantly inhibited CH_4 emissions (up to 98%) but severely impaired GP and VFA concentrations, indicating excessive antimicrobial activity. In contrast, low doses (50 mg/g DM) exerted limited antimethanogenic effects with minimal effects on VFAs. In Experiment 2, garlic alone (G100) showed strong antimethanogenic effects (–70 to –78% CH_4) while preserving overall fermentation and promoting a shift toward propionate and butyrate on day 1. Garlic-based blends (G50–C50 and G75–C12–O12) sustained CH_4 reduction (76% and 86% respectively) while maintaining GP and VFA concentrations. In contrast, cinnamon alone (C100) and oregano alone (O100) strongly suppressed CH_4 (up to –90%) and ammonia-N (up to –40%) but markedly impaired fermentation. Bliss independence analysis confirmed the synergistic interactions among garlic-based combinations for CH_4 reduction and propionate enhancement above the expected values. The incubation during 5 consecutive days amplified the antimethanogenic effects of all additives, likely due to accumulative microbial inhibition, however garlic was the only that maintained fermentation activity overtime. The ternary blend G75–C12–O12 provided the best long-term balance between CH4 mitigation and fermentative stability. Overall, garlic extract demonstrated a robust antimethanogenic potential while preserving fermentative activity in vitro. Nonetheless, potential issues related to palatability and odor transfer to milk may arise under practical conditions. Combining garlic extract with cinnamon and oregano (G50-C50 and G75-C12-O12) enabled to decrease the garlic dose while increasing mitigation efficacy without impairing rumen fermentation. The combined use of ANOVA and Bliss proved to be a valuable approach for investigating interactions among feed additives. These findings warrant further in vivo validation to determine their practical feasibility.

In vitro screening of succinogenic bacteria and dicarboxylic acids as alternative electron sinks during bromoform-mediated methanogenesis inhibition

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Combining alternative electron sinks with methanogenesis inhibitors may redirect reducing equivalents toward propionate formation when methane (CH_4) production is suppressed, thereby conserving energy otherwise lost as CH_4. A randomized complete block design was conducted to evaluate the effects of a CH_4 production inhibitor (oil-stabilized bromoform ($CHBr_3$); 4,000 mg $CHBr_3$/kg oil; Rumin8 Pty Ltd, Australia), alone or in combination with either succinogenic bacteria (Native Microbials, Inc., San Diego, CA, USA), fumarate, or malate (Bartek Ingredients Inc., Stoney Creek, ON, Canada) on gas production and composition, digestibility and fermentation parameters in an in vitro batch culture system. Rumen fluid was collected from two cannulated beef heifers fed a diet containing 90% concentrate and 10% forage (dry matter (DM) basis), which also served as the incubation substrate. The experiment was conducted in three independent runs (blocks), with sampling at five time points (3, 6, 12, 24, and 48 h). Within each run, duplicate vessels (n = 2) were allocated to each treatment at each time point, yielding a total of six replicate vessels per treatment per time point. Treatments consisted of: (i) a control without additives (CTL); (iii) oil-stabilized $CHBr_3$ at 2% of dietary DM (BR); and (iii) BR combined with either succinogenic bacteria (BACT), or fumarate at 0.5% (FLOW), 1.5% (FMED), or 2.5% (FHIGH) of substrate's DM, or malate at 0.5% (MLOW), 1.5% (MMED), or 2.5% (MHIGH) of substrate's DM. All additives (except $CHBr_3$) were diluted in anaerobic buffer, and 1 mL of the corresponding solution was added to each incubation vessel at the start of incubation; anaerobic buffer alone was added to CTL and BR. Data were analyzed using linear mixed-effects models (lmer; RStudio), with treatment, time, and their interaction as fixed effects and block as a random effect. Treatments MLOW and MHIGH decreased pH relative to remaining treatments ($P \leq 0.01$). No treatment effects were detected for DM or neutral digestible fiber digestibility ($P \geq 0.12$). Inclusion of $CHBr_3$, for all the treatments, decreased CH_4 production below the limit of detection and increased aqueous hydrogen (H_2) concentration across all $CHBr_3$-containing treatments compared with CTL ($P \leq 0.01$). However, gas phase H_2 concentration was not affected by treatments ($P \geq 0.35$). Addition of CHBr3 decreased acetate and increased ethanol and formate concentrations compared with CTL ($P \leq 0.05$). Propionate, butyrate, succinate, isovalerate, and valerate were not affected by treatment ($P \geq 0.25$). Isobutyrate, 2-methylbutyrate, and lactic acid were below the limit of detection across all treatments. Bromoform supplementation decreased CH_4 production and shifted fermentation away from acetate toward ethanol and formate production, suggesting microbial adaptation to methanogenesis inhibition. However, under the conditions of this experiment, adding BACT, fumarate, or malate did not effectively redirect reducing equivalents toward increased propionate production.

In vitro ruminal digestibility of bay laurel (Laurus nobilis L.) essential oil at different concentrations

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The global rise of antimicrobial resistance, linked to antibiotic use in human medicine and intensive livestock production, has increased interest in sustainable strategies to reduce antibiotic reliance in animal production. In this context, plant-derived bioactive compounds have attracted attention as complementary approaches to conventional antimicrobials. Although essential oils cannot replace therapeutic antibiotics, their use as phytogenic feed additives may help modulate rumen fermentation and improve feed utilization efficiency. However, identifying appropriate supplementation levels remains challenging, as excessive dosages may negatively affect ruminal microbial balance and impair ecosystem functionality. The aim of this study was to evaluate the effects of bay laurel essential oil supplementation at different concentrations on in vitro digestibility. Bay laurel essential oil was extracted from leaves of Laurus nobilis L., mechanically separated from branches, dried in a ventilated oven, and subsequently subjected to steam distillation for 3.5 h using an Inherba Inox Plus extractor. Essential oil yield was expressed as percentage (w/w), corresponding to the amount of essential oil obtained from 100 g of dried plant material. The extracted essential oil was evaluated at four concentrations (0, 25, 50, and 100 mg/L) to assess its effects on in vitro digestibility. Rumen fluid was collected and stored in thermos flasks as inoculum to start in vitro fermentation, which was conducted for 48 h using the Daisy II incubation system (ANKOM Tech., Fairport, NY). In vitro digestibility was calculated as (PB − PA)/PB, where PB represents the percentage of a given parameter before digestion and PA the percentage of the same parameter after digestion, both expressed on an organic matter basis. Lipids and crude protein were determined according to AOAC procedures. Crude fiber was determined using the Weende method. Neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL) were determined. Gas production was measured using an automated pressure transduction system (ANKOM Technology, Macedon, NY). Data were tested for normal distribution using the Shapiro–Wilk test, and analysis of variance (ANOVA) was performed considering treatment as a fixed effect (SAS, 2018). Post hoc comparisons among means were conducted using Bonferroni test. The analysis showed that essential oil supplementation increased apparent disappearance at 50 mg/L ($P < 0.01$), and reduced it at higher dosage ($P < 0.01$). Also other parameters like gas production and fiber disappearance showed same trend, with higher values at 50 mg/L and reduced values at 100 mg/L ($P < 0.01$). Overall, the results indicate a dose-dependent effect of bay laurel essential oil on in vitro ruminal fermentation. At lower concentrations, supplementation appeared to be associated with improved digestibility parameters, suggesting a favourable modulation of microbial activity and more efficient substrate utilization. Conversely, increasing doses, especially at the highest concentration, were associated with reduced digestibility and lower gas production. These findings suggest the existence of a dosage cut-off beyond which the effect shifts from potentially beneficial to detrimental, in agreement with previous literature. Further research is needed to clarify the long-term effects of essential oil supplementation and to determine the extent to which it may influence metabolism and energy partitioning in ruminants.

How carbonate based buffers and magnesium based buffers affect methane production in a rumen simulated environment

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Rumen buffering and methane (CH_4) emissions are key factors influencing both digestive health and environmental sustainability in intensively fed beef and dairy cattle. High-grain diets, commonly used to maximise energy intake, can disrupt rumen pH homeostasis, leading to acidosis and reduced animal performance. Effective rumen buffering strategies including targeted dietary interventions and feed additives help maintain optimal pH and support beneficial rumen microbiota, enhancing nutrient utilisation and overall productivity. However, improved rumen fermentation efficiency can also increase CH_4 output, a potent greenhouse gas responsible for approximately 40% of global anthropogenic emissions from livestock. Balancing rumen health with emission reduction remains a critical challenge for sustainable animal agriculture. This study aims to identify how commercially available carbonate and Mg based rumen buffers affect methane production in an in vitro rumen fermentation model. Three feed grade buffer sources (1 x carbonate and 2 x Mg based) with particle size ranging from 289-339µm (Dx95) were obtained from commercial sources with each buffer being assessed for its total Mg content as well as its Mg solubility. Each Ankom RF rumen simulation vessel contained a buffer treatment (8.66mg) and a 20% starch dairy diet (4g) to which 40 ml of fresh rumen fluid from a Friesian cow was added which was subsequently incubated at 39$_o$C for 12 h. Vessels were analysed after 6 and 12h with solubilised Mg, pH, methane and VFA production measured. Mg based buffers used in this study ranged from 42-52%w/w Mg. Solubility was dependent on fermentation time with higher Mg^{2+} solubilised at 12 hours (44-65%) versus 6 hours (18-52%). However, differences in Mg solubility between samples was only significant ($p>0.05$) at 6 hours. All buffers kept pH above pH 5.5 after 6 hours of fermentation with the 52% soluble Mg buffer having the highest average area above pH 5.8 (2.07 mmol H^+.s) and the 18% soluble Mg buffer having the lowest average area above pH 5.8 (0.7 mmol H^+.s). The carbonate buffer-maintained pH within an optimal average area above 5.8 (1.5 mmol H^+.s). Methane production between buffers was significantly different at 6 hours ($p>0.05$) with high soluble Mg buffer producing significantly more (0.36g CH4) than the carbonate buffer and low solubility Mg buffer (0..24 and 0.23 g, respectively). This study provides greater understanding on how rumen buffers can alter fermentation dynamics leading to differences in CH4 production. The data suggests that highly soluble magnesium buffers may increase methane production due to rumen pH being higher than optimal.

Effect of hydroponic barley fodder-containing diets on methane production and rumen fermentation in dairy cattle: an in vitro assessment

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Water and arable land limitations, coupled with the urgent need to reduce enteric methane (CH4) emissions from the livestock sector, represent a major challenge to modern animal production systems. Hydroponic barley fodder (HBF) offers a resource-efficient, "landless" feed alternative, though its specific ruminal fermentation kinetics and methane mitigation potential remain largely uncharacterized. The objective of this study was to characterize the nutritional value and evaluate the enteric CH4 mitigation potential of HBF at two distinct harvest maturities when incorporated into cattle diets, validating its use in sustainable, land-independent ruminant production systems. The HBF was collected on two different days of the growth cycle, on day 4 (T4: peak enzymatic activity and energy mobilization) and day 6 (T6: standard harvest maturity and maximum biomass) as critical metabolic inflection points. HBF was tested as a single ingredient (100% HBF) and in combination with hay and concentrate at different inclusion levels (from 0 to 60%). In vitro fermentation was assessed utilising the Hohenheim Gas Test (HGT) technique. Individual rumen fluid from three fistulated cows was mixed with a buffer solution, and 200 mg of total substrate was incubated in glass syringes for 24 hours at 39 °C to measure pH, gas volume, gas composition, ammonia and digestibility. We hypothesized that HBF, being nutrient-dense and lower in fiber than standard hay, would favor propionate production (a hydrogen sink), thereby lowering methanogenesis. To definitively validate this metabolic shift, we evaluated the CH4/CO2 ratio (expressed as mL/L) and sampled the ruminal fluid to analyze Volatile Fatty Acid (VFA) profiles. While VFA analytical results are currently pending, the CH4/CO2 ratio serves as a crucial preliminary indicator: a lower ratio confirms a genuine shift towards hydrogen-consuming pathways, proving that CH4 mitigation is achieved through improved energetic efficiency rather than merely reflecting a decrease in overall feed digestibility. Here, we show preliminary findings that align with our expectations. The pure substrates exhibited notably higher digestibility up to 71.6% and lower CH4/CO2 ratios (reduced by up to 6.8%) compared to the control diet (60% DM hay, 40% DM concentrate), furthermore, we observed a progressive dose-response effect in the hay-substitution diets. The observed difference in CH4 mitigation potential between the harvesting stages suggests that the physiological maturity of T6 optimizes ruminal fermentation characteristics. Hence, our next step is to conduct final verification runs and integrate the pending VFA data to consolidate these promising gas kinetics and digestibility metrics. Preliminary in vitro data indicate that HBF is a highly digestible feed component with the potential to reduce the CH4 emissions. Therefore, replacing fibrous roughage with specific harvest maturities of hydroponic fodder is a promising strategy but needs to be verified in following in vivo studies.

Barrier protective effect of single-malt distillery-derived Lactobacillus postbiotic

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The gut barrier, a multilayered system of cells, mucus, proteins, and microbes, plays a vital role in preventing pathogen infiltration, making it central to global efforts in infectious disease prevention and research. The objective of this study was to evaluate the effects of Lactobacilli postbiotic formulation, developed using a patented process that repurposes distillery by-products, on inflammation and gut barrier integrity. Murine macrophage RAW264.7 cells were treated with the postbiotic and were subsequently primed with lipopolysaccharide (LPS) to induce a pro-inflammatory response. The inflammatory response was assessed by measuring the expression of TNF-α mRNA by rtPCR. Additionally, intestinal epithelial Caco-2 cells cultured as monolayers in transwells were exposed to Escherichia coli to induce barrier disruption. Subsequently the monolayers were treated with the postbiotic. Barrier integrity was evaluated by measuring FITC-Dextran (MW: 3 kDa) permeability across the monolayer and analysing tight junction gene expression. Results demonstrated that postbiotic activated RAW264.7 cells under unprimed conditions, as evidenced by increased TNF-α levels. In contrast, LPS-primed cells treated with postbiotic demonstrated reduced expression of TNF-α suggesting anti-inflammatory activity. In Caco-2 cells, E. coli exposure increased FITC-Dextran permeability. The postbiotic treatment significantly ($P<0.05$) mitigated this effect with >50% reduction in permeability and caused a significant ($P<0.05$) downregulation of CLDN-2 tight junction protein expression with a fold change of 0.15. Overall, the findings suggest that postbiotic possess immunomodulatory properties and contribute to the restoration of epithelial barrier function, highlighting their potential role in reducing tissue damage associated with infection leading to gut health maintenance. This represents a unique advancement in postbiotic development through the sustainable and innovative use of distillery by-products.

Milk oligosaccharides shape the gut mucosal barrier in vitro in suckling rabbits

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Milk oligosaccharides (MOs) are non-nutritional complex bioactive carbohydrates that are naturally present in mammalian milk. In rodent and human newborns, they are known to exert multiple beneficial functions in the intestinal tract, namely preventing pathogen adhesion, favoring the growth of beneficial bacteria through prebiotic action, enhancing epithelial barrier function and having immunomodulatory capacities. Given their beneficial effects, MOs could represent promising novel feed additives in livestock nutrition, particularly to facilitate the weaning transition. Our study aimed to investigate in vitro the effects of three structurally different types of MOs: a neutral MO, represented by lacto-N-tetraose (LNT), a fucosylated MO, represented by 2'-fucosyllactose (2'FL) and a sialylated MO, represented by 6'-sialyllactose (6'SL), on the gut mucosal barrier using the rabbit, a livestock species particularly susceptible to digestive disorders around weaning. Two gut mucosal barrier components were studied: the gut epithelium and the immune cells in the underlying lamina propria. Suckling rabbit cecal epithelial cell monolayers were derived from organoids to reproduce the cellular complexity of the intestinal epithelium (n=4). Gut immune cells were studied through the culture of suckling rabbit cecal lamina propria mononuclear cells (LPMCs) that comprise most gut immune cells, mainly macrophages, dendritic cells and lymphocytes (n=18). Cells were cultured in vitro under control conditions or exposed to LNT, 2'FL or 6'SL (20 and 2 mg/ml respectively for epithelial cells and LPMCs). The gene expression of 84 genes implicated in epithelial barrier function, inflammatory and immune response was assessed after 24 h of culture. All MOs induced a marked upregulation of epithelial cell differentiation (KRT20, CA2) but had no impact on epithelial cell proliferation (PCNA, MKI67). Regarding the barrier function, the three MOs induced a marked decrease in the expression of genes coding for transmembrane mucins (MUC1, MUC13). While 2'FL had no impact on tight junctions, and LNT solely reduced the expression of TJP2, 6'SL induced a moderate reduction in the expression of two key tight junction markers (TJP1, CLDN3) in accordance with a reduction in transepithelial resistance. Finally, MOs impacted inflammatory pathways in epithelial cells with a sharp increase in CXCL10 expression with LNT and 6'SL and a 10-fold increase in the expression of CXCL8 solely with 6'SL. In LPMCs, MOs had no impact on proliferation (MKI67) or lymphoid (CD69, FOXP3, JCHAIN) and myeloid (CD14, CD80, CD86, FLT3) cell specific markers, regardless of MO structure. Inflammatory pathways were however modulated by MOs in LPMCs, with a consistent upregulation of inflammatory markers (IL6, CXCL8) with 2'FL and 6'SL and a marked upregulation of regulatory pathways (IL10, IL2RA) and inflammatory marker CCR5 solely with 2'FL, while LNT had no effect on LPMCs in vitro. Our study demonstrates an impact of MOs on the gut barrier function and highlights a structure specificity. However, a greater impact of MOs on epithelial cells compared to LPMCs was evidenced, that could be associated with the greater physiological exposition of these cells to MOs in vivo. Interestingly, in both cell types, 6'SL induced a marked increase in CXCL8 indicating clear immunomodulatory impacts of this MO that should be further studied. Co-culture models would enable to better understand the direct and indirect impacts of MOs on the different components of the gut mucosal barrier. Finally, these results pave the way for future on-farm applications that should be evaluated in in vivo studies.

Curcumin loaded liposomes ameliorates DSS-induced colitis in mice: in vitro and In vivo studies

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Inflammation is a significant health concern in livestock animals. It adversely affects animal health, compromises performance, and reduces final product quality. Curcumin, a polyphenolic compound derived from the rhizomes of the turmeric plant, exhibits anti-inflammatory properties. However, its application is limited by poor bioavailability. Nano-encapsulation enhances its physicochemical stability and biological activity, thereby improving its efficacy compared with free curcumin. In this study, we synthesized curcumin-loaded liposomes (Cur-L) and evaluated their protective effects against inflammation using in vitro and in vivo models. Cur-L showed an average particle size of 172.5 ± 3.0 nm and a zeta potential of −29.42 ± 1.32 mV, along with high encapsulation efficiency (98%) and drug-loading capacity (9.8%). The protective effects of Cur-L against inflammation were confirmed in LPS-induced RAW 264.7 cells and a DSS-induced mouse model of colitis. Cur-L decreased the nitric oxide production in LPS-stimulated RAW 264.7 cells. In vivo, Cur-L administration alleviated DSS-induced clinical symptoms, including body weight loss and disease activity index scores, and improved colon length and histopathological damage. Additionally, Cur-L modulated the gut microbiota by increasing the abundance of beneficial bacteria. Together, these results indicate that Cur-L is a potential therapeutic agent for treating inflammation and warrants further investigation. Keywords; Curcumin, DSS-induced colitis in mice, Inflammation, livestock animals

Ensiled forages modulate rumen fermentation and microbiome in lambs

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Ensiled forages are essential to feed ruminant animals during shortage of either fresh or dried forages. Ryegrass (RG) is mostly ensiled to fill the gap of forage shortage especially during cold or dry seasons. Indeed, many other forages such as whole crop cereals and legumes are also ensiled for feeding ruminants. Interestingly, ensiled Brassica (BR) forages are rarely used as a novel alternative when availability of ensiled RG is restricted. Therefore, a series of replicated field, animal and lab-studies evaluated the potential value of ensiled BR against RG for lambs. Nevertheless, this paper presents only their nutrient composition and fermentation plus microbiome before and after in vitro incubation with rumen fluid (RF) from freshly killed lambs. The BR had (g/kg) significantly ($P<0.01$) lower DM (192 vs 660), pH (3.5 vs 6.1) and NDF (384 vs 485) but higher CP (216 vs 135), DUP (53 vs 21) and NH3 (58 vs 46) than RG. Representative samples of RF were collected immediately post-killing from 24 Mule lambs weighing 38kg (+1.6) consuming ensiled BR or RG. The RF samples were pooled to get 4 replicated RF per group of BR and RG consuming lambs. The pooled samples were filtered (unbuffered, RF1) before aliquots of RF1 were buffered (RF2) for in vitro incubation with samples of BR and RG to determine their respective in vitro dry matter degradability (IVDMD). Aliquots of post-incubated RF2 were filtered (RF3) and tested alongside aliquots of RF1 and RF2 for rumen fermentation but only RF1 and RF3 were used to extract DNA to study microbiome. The DNA samples were subjected to the Shotgun Metagenomic analysis with Next Generation Sequencing and Bioinformatics by Novogene Ltd. The results were either numerically or visually illustrated for similarities or variations in RF1 and RF3 for RG and BR. Mean IVDMD (g/kg) of BR at 763 was significantly greater than 525 for RG (SEM 13, $P<0.01$). Mean pH was higher at 7.9 for RF1 than 6.9 for RF3. Moreover, RG showed a numerically higher pH of 7.9 than 7.7 for BR. RF1 had significantly higher NH3 of 361 mg/L than RF3 at 294 mg/L. Total VFA of BR at 39.6 mmol/L were more than RG at 34.6 mmol/L. The main effects of BR on NH3 and total VFA were also higher than RG. Metagenomic results showed comparable total scaftig (>500bp) number (344,455 vs 352,920) and average length (1,013 vs 1,040 bp) in RF1 of RG and BR respectively. Conversely, F3 showed 268,191 and 219,022 scaftigs with mean length of 1,088 and 1,032bp for RG and BR respectively. Overall, the RG and BR varied for identical and unique genes. The ensiled forages compared well for the abundance of bacterial phylum, family, genus and species. The phylum data showed good correlation ($r > 0.6$) and the alpha diversity indices compared well for both forages. However, in vitro incubations with RF3 decreased some phyla (Bacteroidetes), families (Prevotellaceae), genera (Prevotella) and species (Prevotella). Conversely, it increased some phyla (Firmicute), families (Lachnospiraceae), genera (Ruminococcus) and species (Selenomonas ruminantium & bovis). The Bacteroidetes phylum was more common for RG than their BR counterparts. These variations reflected the higher fibre content in Ryegrass than Brassica. The enhanced abundances of Firmicutes, Bacteroidetes and Fibrobacteres phyla indicated perhaps the improved fibrolytic and proteolytic activities in RF with RG. The ACE and Cha1 indices and phylum diversity indicated that the RG based RF1 and RF3 had higher phylum richness than their BR counterparts. Apparently, ensiled Brassicas can be used to not only replace or complement Ryegrass but also modulate rumen fermentation and microbiome in lambs. This strategy may optimise nutrient use efficiency to promote sustainable ruminant production.

Linking in vitro methane yield with rumen fermentation patterns and milk quality traits during the transition period

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Enteric methane (CH_4) mitigation in dairy systems requires scalable indicators capturing biologically meaningful variation among cows under farm conditions. While in vitro CH_4 yield is widely used to characterize feed and additives for ruminants, the potential of in vitro systems to assess the methanogenic potential of rumen fluid, and the extent to which these measurements reflect milk quality traits and rumen metabolism across the transition period, remains unclear. We hypothesized that variation in in vitro CH_4 yield could reflect rumen metabolic phenotypes associated with milk fatty acid (FA) profiles and fermentation dynamics during the transition period. Sixty Holstein–Friesian cows were sampled at the beginning of the dry period (T0, 60 d prepartum) and early lactation (T1, 30 d postpartum). Individual rumen fluid samples were collected using an oro-ruminal probe. A subsample was analyzed for volatile fatty acids (VFA) and ammonia (NH_3), while the remaining fluid was used as inoculum for in vitro fermentation assays to evaluate gas production kinetics and CH_4 yield per gram of total mixed ration (TMR). Milk samples were analyzed for FA composition by gas chromatography. Cows were equipped with rumen boluses to monitor rumen pH and rumination activity. CH_4 yield was also predicted from milk FA using published equations. Within each sampling point, cows were classified into low, medium, and high in vitro CH_4 yield tertiles (T0: <24.1, 24.1–26.8, >26.8; T1: <21.2, 21.2–24.6, >24.6 mL/g DM). Data were analyzed separately for T0 and T1 using linear models including CH_4 class, parity order, and parity season as fixed effects. Response variables included rumen fermentative parameters (pH, VFA, NH_3), milk yield, milk FA profile and FA-based CH4 predictions. Variation in in vitro CH_4 yield was associated with rumen parameters measured in vivo. At T1, cows in the low CH_4-yield tertile showed lower rumen pH measured in vivo ($P < 0.05$), higher propionate concentrations, and a lower (acetate + butyrate)/propionate ratio ($P < 0.05$), consistent with a more acidic rumen environment and fermentation patterns typically associated with reduced methanogenic activity during early lactation. This pattern was not observed during the prepartum stage (T0). Late-phase in vitro gas production increased across CH_4-yield tertiles at both stages, although overall volumes were lower at T1, suggesting stage-dependent differences in fermentative activity across the transition period. Clearer differentiation of milk FA emerged at T1. In early lactation, cows in the low CH_4-yield tertile showed higher total milk and n-6 PUFA contents ($P <0.05$), whereas high CH_4-yield cows showed relatively higher C4:0 and C16:0 contents. In contrast, prepartum patterns were less pronounced, although odd-chain FA (OCFA) increased with CH_4 yield at T0 ($P <0.10$). This relationship reversed at T1, where OCFA decreased progressively from low to high CH_4-yield cows ($P <0.05$). FA-based CH4 predictions were consistent with measured in vitro CH_4 classes but only at T1. These stage-dependent shifts indicate that associations between CH_4 phenotype and rumen-derived milk lipid markers vary across the transition period. Overall, in vitro CH_4 yield captured biologically meaningful between-cow variation in rumen fermentation patterns and milk lipid composition, supporting further investigation into its potential for scalable identification of low-emission phenotypes in commercial dairy systems.

Extracellular vesicles from low- and high-virulence Streptococcus uberis strains differentially regulate bovine neutrophil viability and inflammasome activation in vitro

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Streptococcus uberis is a major causative agent of bovine mastitis; however, the role of bacterial extracellular vesicles (BEVs) in strain-specific modulation of host innate immunity remains poorly understood. Using a previously optimized and MISEV 2023-compliant workflow for BEV isolation, this study evaluated whether BEVs derived from two S. uberis strains with low or high virulence exert differential effects on bovine polymorphonuclear cells (PMNs). BEVs were isolated from bacterial culture supernatants by sequential centrifugation, 0.22 µm filtration, and ultracentrifugation. Vesicle preparations were characterized by nanoparticle tracking analysis, cryo-electron microscopy, Western blotting, and flow cytometry to confirm particle size distribution, morphology, and Gram-positive membrane origin. For functional assays, BEVs were normalized based on total protein content, and three protein concentrations for each preparation were tested. PMNs were isolated from bovine peripheral blood and exposed to increasing BEV doses to generate dose-response curves and determine median lethal dose (LD50) values using the WST-1 assay. Cell viability and proliferative capacity were assessed by measuring metabolic activity, while inflammatory activation was evaluated by measuring inflammasome-associated caspase-1 activity as a marker of pyroptotic signaling. BEV exposure induced concentration-dependent effects on PMN viability and inflammasome activation. BEVs derived from the high-virulence strain caused a more pronounced reduction in neutrophil viability and stronger caspase-1 activation at lower protein-normalized concentrations compared with vesicles from the low-virulence strain, indicating strain-dependent immunomodulatory properties. These findings integrate standardized BEV isolation procedures with functional immune assays to quantify vesicle bioactivity on bovine neutrophils. The data support the hypothesis that differences in S. uberis virulence may be partially mediated by BEV-driven modulation of neutrophil survival and inflammatory cell death pathways, providing mechanistic insight into host-pathogen interactions during bovine mastitis. Keywords: bovine mastitis, bacterial extracellular vesicles, inflammation, cell death, Streptococcus uberis.

A botanical and organic acid blend protects intestinal cell viability and reduces heat stress-induced oxidative stress across human, porcine, and avian in vitro models

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Heat stress (HS) is a major challenge in animal production, with rising global temperatures increasing its economic and welfare impact. At the intestinal level, HS disrupts epithelial barrier integrity, increases reactive oxygen species (ROS) production, and promotes cell death, ultimately impairing nutrient absorption and immune function. Dietary strategies capable of protecting intestinal cells from HS-induced damage are therefore of practical relevance for both swine and poultry production. The aim of this study was to evaluate the protective effect of a blend of botanicals and organic acids (AviPlus®, AP), against HS-induced viability loss and oxidative stress, using three intestinal cell lines: human Caco-2, porcine IPEC-J2, and avian cIEC-H2. Cell viability was assessed under two experimental designs. In the concurrent treatment design, AP was added at HS onset (42°C, 24h) and maintained for 6 days, to evaluate both protection during and recovery after the challenge. In the pre-treatment design, cells received AP for 6 days before HS exposure (42°C, 24h), with viability recorded at day 8. AP was used at a final concentration of 1000 ppm. Viability was measured with PrestoBlue® (Thermo Fisher Scientific). For ROS assessment, cells were pre-treated for 6 days and then exposed to HS for 4h at 42°C. ROS were quantified with CellROX® (Thermo Fisher Scientific). Viability data were analyzed by two-way ANOVA (time × treatment) and ROS by one-way ANOVA with Tukey's post-hoc test. In the concurrent treatment design, HS reduced viability to 49% (cIEC-H2) and 45% (IPEC-J2) of thermoneutral control by day 6. AP significantly limited this drop (cIEC-H2 76%, IPEC-J2 90%; $p<0.05$ vs HS control). In Caco-2, HS reduced viability to 83% at 24h, while AP-treated cells maintained 89% and subsequently recovered beyond thermoneutral levels (>110%) by day 6. In the pre-treatment design, AP improved post-challenge viability across all three cell lines: IPEC-J2 102% vs 81% (HS control), cIEC-H2 90% vs 80%, Caco-2 88% vs 81% (all $p<0.05$). HS significantly increased ROS levels compared to thermoneutral control across all three cell models: +28% in Caco-2 and cIEC-H2, +82% in IPEC-J2. AP reduced HS-induced ROS by approximately 13% in Caco-2, 15% in cIEC-H2, and 22% in IPEC-J2 (all $p<0.05$ vs HS control). In Caco-2 and cIEC-H2 this corresponded to a full restoration of thermoneutral levels, while in IPEC-J2 the reduction was significant but partial. AP consistently reduced HS-induced viability loss and oxidative stress across all three intestinal cell models. The protective effect was observed under both concurrent and preventive administration, suggesting that the blend may exert its action through multiple mechanisms. The consistent response across species supports its potential as a nutritional strategy to maintain intestinal health and animal performance under heat stress conditions in swine and poultry production.

Exploring the combined effects of plant extracts, tannins, and saponins on cecal fermentation in weaned piglets using consecutive batch culture incubations

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The post-weaning period in piglets is characterized by abrupt dietary changes, reduced nutrient digestibility, and instability of the gut microbiota, frequently leading to digestive disorders. Therefore, nutritional strategies aimed at enhancing hindgut fermentation are a priority during this critical period. Plant extracts such as essential oils, tannins, and saponins have attracted increasing attention due to their antimicrobial and immunomodulatory properties. Although these compounds have shown promising effects when supplemented alone, their potential synergistic effects of their combinations in weaned piglets remain largely unexplored. The objective of this in vitro study was to evaluate the individual and combined effects of dietary supplementation with: a plant extract blend containing turmeric, green tea and Scutellaria baicalensis (P); tannins from chestnut and quebracho (T); and saponins from yucca, quillaja, and oats (S), on cecal fermentation in weaned piglets. The experimental diet was subjected to enzymatic digestion with HCl, pepsin, and pancreatin to simulate gastric and small intestinal digestion. The undigested residues were then incubated in batch culture system using four pooled cecal inoculum from 40 weaned piglets. A total of 11 treatments were evaluated: a control (CTL); individual additives at 5% inclusion rate (P100, T100, S100); binary combinations (P50–T50, P50–S50, T50–S50); and ternary combinations (P33–T33–S33, P75–T12–S12, P12–T75–S12, P12–T12–S75). Fermentation bottles were incubated anaerobically at 37 °C. Incubations lasted five days, with daily renewal of the incubation medium. Gas production (GP), methane and fermentation products were measured on days 1 and 5. On day 1, cecal pH and total volatile fatty acid (VFA) concentration were not affected by additive supplementation, indicating no inhibition of overall hindgut fermentation. However, all additives reduced ammonia-N concentration (up to 5%, $P < 0.001$) and the molar proportion of branched-chain fatty acids (−7%, $P = 0.008$), suggesting reduced proteolysis compared with CTL. This effect was particularly evident in treatments containing tannins (T100, P12–T75–S12, P50–T50, and T50–S50). Treatment P100 tended to increase gas production (+7.9%, $P = 0.087$) and significantly increased the molar proportion of butyrate (+4.8%, $P = 0.029$) compared with CTL. These effects were consistent when fermentation products were expressed per unit of substrate. Additionally, treatment P75–T12–S12 showed a tendency to increase propionate production. By day 5, most previously observed effects were maintained and, in some cases, intensified, likely reflecting the antimicrobial properties of the additives. Treatment T100 reduced total VFA concentration compared with CTL (−25%, $P < 0.001$), although this negative effect was attenuated when tannins were combined with other additives. The inclusion of low doses of tannins in ternary combinations (P33–T33–S33, P75–T12–S12, and P12–T12–S75) reduced proteolysis without compromising overall fermentation activity and increased the molar proportion of butyrate ($P < 0.001$). In conclusion, this in vitro study demonstrates that combining plant extracts, tannins, and saponins may represent a promising nutritional strategy to optimize hindgut fermentation in weaned piglets. Low-dose tannin inclusion in combination with plant extracts and saponins reduced proteolysis while enhancing the production of beneficial VFA. Further in vivo studies are required to confirm these findings.

Dose-Response Effects of Isoacids Supplementation in Dry Cow Diets: Impacts on Microbial Nitrogen and Fiber Fermentation

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Recent research has demonstrated the benefits of isoacids supplementation in enhancing rumen efficiency, notably increasing microbial protein synthesis and fiber fermentation in lactating dairy cows (Firkins et al., 2024; Redoy et al., 2025). However, the dose-response relationship of isoacids when supplemented in dry cow diets, particularly in relation to requirements defined by the CNCPS model, remains unexplored. This study investigated the effects of incremental isoacid supplementation on microbial growth and fiber fermentation in dry cow diets, including dosages exceeding established model requirements. Four dual-flow continuous fermenters were assigned to an experiment with a 4×4 Latin Square design, each fed a dry dairy cow diet formulated using the CNCPS model within NDS software. The diet (% DM) was comprised of corn silage (34.4), wheat straw (29.3), grass silage (14.0), soybean meal (11.7), fine-ground corn grain (10.4), and mineral mix (0.2), with an initial isoacid deficit of 0.5 g/kg DM. Treatments included: control (CON), low (LIA, 0.8 g/kg DM), medium (MIA, 1.2 g/kg DM), and high (HIA, 1.6 g/kg DM) isoacids dosages. Each period involved a 7-day adaptation and 5-day sampling phase. Liquid and solid dilution rates were set at 10%/h and 5%/h, respectively. Samples were collected at 9 and 24 hours post-feeding for microbial nitrogen (Kjeldahl method), neutral detergent fiber (NDF), and acid detergent fiber (ADF) analyses. Data were analyzed using the lme4 package in R, with treatment and period as fixed effects and fermenter nested within period as a random effect; linear, exponential, and quadratic models were assessed. At 9 hours post-feeding, microbial nitrogen concentrations did not differ significantly among treatments. NDF digestibility (NDFd) exhibited a quadratic trend ($p < 0.1$), and ADF digestibility (ADFd) followed a linear pattern ($p < 0.1$), but these were not mirrored by nitrogen efficiency measures. At 24 hours, microbial nitrogen concentration increased linearly with isoacids dosage, with each unit increase yielding 37 mg N/L more microbial nitrogen ($p < 0.01$). No significant trends were observed in fiber fermentation parameters at this time point, but nitrogen efficiency improved linearly with increased isoacid supplementation ($p < 0.05$). The delayed response between fiber digestibility and microbial nitrogen enhancement is hypothesized to result from the lower digestibility of fiber sources like wheat straw in dry cow diets compared to lactating cow diets. The findings indicate that isoacid supplementation of dry cow diets promotes a linear increase in microbial nitrogen production, even at dosages exceeding CNCPS model requirements. While fiber fermentation is enhanced, NDF digestibility appears to plateau at higher supplementation levels. These results suggest that isoacid supplementation may improve metabolic efficiency in prepartum dairy cows, offering potential benefits for preparation for calving and transition into lactation.

The effects of supplementation with the natural additive Fator P on feeding of Rhipicephalus microplus parasite larvae.

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Infestations by the hematophagous ectoparasite R. microplus are one of the main challenges to health of beef and dairy cattle. Fator P is a natural zootechnical feed additive that has been shown to improve several production features in beef and dairy cattle. Due to its composition and to the mechanisms by which ticks infest and feed on their hosts, we hypothesised that when cattle receive Fator P in the diet, can have a negative impact on the rate of engorgement by larvae of R. microplus. In order to test this hypothesis, we employed an artificial feeding system (AFS) with unfed infesting larvae to engorge them on fresh blood supplied from bovines fed, or not, with the Factor P additive for different periods of time. Purebred, castrated Holstein steers, aged 18 to 20 months were randomized into two groups based on weight, body condition score, paternity and innate tick loads. The study was blinded and both groups were confined in individual pens in a feed lot with an empty pen separating each animal; the test group received Fator P (dosed at 6,0 gr / animal / day) and both groups received 2,2% of body on a dry matter (DM) in diet. Blood samples were collected in CaCl2 on days 28, 56, 63, 70, 87, and 119 after the start of treatment with the additive; blood and larvae were distributed in triplicate in the AFS for 48 hours . The statistical method used was the Mann-Whitney test, with p-values <0.05. Of the initial total of unfed larvae released in the AFS, the percentage of engorged larvae recovered after 48 hours varied between 21.3% to 24.1% on blood from animals treated with Fator P plus feed, and 39.8% to 39.9% on blood from animals treated with feed alone from days 56 and 119 ab initio treatment. We conclude that when animals feed on natural additive Fator P supplemented meal, it was observed reduction of 40.65% relative to the negative control (p<0.0001) and also the number of engorged females recovered on the animals in the same experiment (data not shown). In the field, the results showed that the inclusion of the additive in the animals' diet was able to reduce tick counts by up to 34.0% (p=0.2429) in animals after 42 days of treatment, and by 74.4% (p=0.0024) in animals after 98 days of treatment. These findings corroborate our field observations and suggest that the additive interferes with the physiology of the tick. Others has shown that guinea pigs fed on fish oil contain high levels of eicosapentaenoic acid, which impairs accumulation of arachidonic acid in salivary glands and subsequent production and secretion of dienoic prostaglandins in tick saliva. These salivary mediators are essential for feeding performance because they increase the amount of blood present in the feeding lesion. Fator P can be an important strategic tool in the control of R. microplus, especially when used in an integrated manner with other control practices.

Effect of fermentation of field pea grains with Companilactobacillus kimchii on the microbiome in pigs using metagenomics and metaproteomics

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Feed fermentation is a processing technique able to increase nutrient digestibility and reduce antinutritional factors in feedstuffs. Additionally, by supplying the gut with prebiotics and probiotics, fermented feed may influence the composition of the gut microbiota. By applying different meta-omics approaches, not only can the composition of the microbiota be determined, but also, by using metaproteomics, the functionally contributing part of the microbiota. We therefore investigated the influence of the fermentation of pea grains on the ileal and faecal microbiome in pigs by using metagenomics and metaproteomics. Four diets based on wheat (20%), barley (13%), rapeseed meal (10%), and 50% pea grains were formulated. The peas were either unfermented (control), unfermented with added lactic and acetic acid, fermented, or fermented with added carbohydrases. The fermented peas were produced by inoculating a mixture of ground pea grains and water (1:1.5, w/w) with 1 ml/kg of an overnight culture of Companilactobacillus kimchii and incubating it at 37 °C for 24 h. Eight barrows (27.6 ± 2.0 kg initial body weight) were fitted with a T-cannula at the distal ileum and assigned to the four dietary treatments in a 4 × 4 double Latin-square design. Daily feed allowance was 4% of the mean body weight. The experiment included 4 experimental periods of 11 days each. Samples of ileal digesta and faeces were collected on the last day of each period for microbiome analyses. For shotgun metagenomics, DNA was extracted using the FastDNA SPIN Kit for Soil (MP Biomedicals LLC, Santa Ana, CA) and sequenced (PE150) on an Illumina NovaSeq 6000 platform (Illumina Inc., San Diego, CA). For protein extraction, bacterial cells were lysed by ultrasonication, and in-gel digestion was performed with trypsin. Metaproteomic analysis of peptides was conducted using an Exploris 480 mass spectrometer with an Ultimate 3000 RSLCnano system (Thermo Fisher Scientific, Darmstadt, Germany). Identification and quantification of protein groups were performed using MetaLab MAG. Statistical analysis was conducted in R. For metagenomics, the median number of reads per sample, which was obtained after quality control and used for downstream analysis, was $34.98 \times 10_6$. Metaproteomic analysis identified ~22,000 protein groups, of which ~17,500 were retained for analysis. In the ileum, β-diversity for both, taxonomic read annotations and microbial proteins, was affected by the dietary treatment ($P < 0.05$), with the fermented diets clustering separately from the other diets. Differential abundance analysis showed an increased abundance for reads and proteins of the genera Companilactobacillus, Weissella, and Enterococcus_B in the ileal digesta for the fermented diets. The ratio of proteins to DNA, calculated from the relative abundances of genera in metaproteomics and metagenomics, respectively, was <1 for Companilactobacillus and Weissella in ileal digesta for the fermented diets, and lower than in the other diets ($P < 0.05$). No effect on β-diversity was detected in the faeces. The results demonstrate that feeding fermented pea grains primarily affected the composition of the ileal microbiota, whereas the effect on the faecal microbiota was less pronounced. However, the discrepancies in relative abundances between metagenomics and metaproteomics point to a lower functional contribution of taxa originating from the fermented pea grains than the taxonomic composition based on metagenomics suggests.

Metabolomic responses of porcine gut organoids to Escherichia coli

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Weaning diarrhea is a common and economically significant disease in young pigs, typically occurring within the first 1–2 weeks after weaning due to stress, dietary changes, and an immature immune system. A primary cause is infection with enterotoxigenic Escherichia coli (ETEC), which adheres to the small intestinal lining via fimbrial adhesins and secretes enterotoxins such as heat-labile (LT) and heat-stable (STb) toxins that disrupt epithelial ion transport, resulting in fluid hypersecretion, diarrhea, dehydration, and impaired growth performance. A deeper understanding of the molecular interactions between ETEC and the intestinal epithelium is essential for the development of improved preventive and diagnostic strategies. In vitro intestinal organoid models provide a physiologically relevant platform to study host–pathogen interactions under controlled conditions. To identify novel metabolic markers associated with early-stage ETEC infection, we investigated metabolite production in porcine gut organoids following bacterial challenge. Organoids were cultured and seeded onto transwell inserts to allow apical infection and basolateral sampling. The response to the ETEC strain CVI-1000 (E. coli O149:K91, F4/K88ac+, LT+, STb+) was compared with strain CVI-1084 (LT+, STb+), which is genetically identical to CVI-1000 except for the absence of the F4 fimbrial adhesin. Cell lysates and basolateral supernatants were collected 2 hours post-infection for metabolomic analysis. Preliminary results demonstrated a significantly higher proportion of adherent bacteria in organoids exposed to CVI-1000 compared to CVI-1084, underscoring the critical role of F4-mediated attachment in early host–pathogen interactions. Ongoing metabolomic analyses aim to elucidate infection-induced alterations in epithelial metabolic pathways and to identify candidate biomarkers for early detection of ETEC-associated weaning diarrhea.

A transcriptomic approach to study the effects of reducing the dietary balanced protein level on small intestine metabolism of slow-growing broilers

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Production of slow-growing broilers has gained interest due to increasing demand for higher welfare standards. However, compared with fast-growing breeds, slow-growing broilers have a greater environmental impact because of poorer feed efficiency. This study investigated the combined effects of reducing dietary metabolizable energy (ME) and balanced protein (BP) on performance, carcass traits, nitrogen balance, carbon footprint and small-intestinal gene expression. A total of 1,008 male Rustic Gold chicks were allocated to 48 pens, balanced for body weight, and randomly assigned to eight dietary treatments (21 chicks/pen; 6 pens/treatment). In a 2×4 factorial design, diets contained either 100% or 98% ME and 100%, 95%, 90% or 85% BP relative to Ross 308 recommendations. Birds received starter (day 0–10), grower 1 (day 10–21), grower 2 (day 21–29) and finisher diets (day 29–42). At day 42, jejunal tissue from two birds per pen was collected for RNA-seq analysis. Gene expression was analysed using DESeq2, with adjusted p-values (Padj) < 0.10 considered significant. Among 13,984 expressed genes, differentially expressed genes (DEG) were identified between birds fed 85% and 100% BP at 100% and 98% ME level (100ME-85BP vs. 100ME-100BP, and 98ME-85BP vs. 98ME-100BP). At 100% ME, only SLC30A2, encoding the zinc transporter ZnT2, was upregulated in 100ME-85 BP compared to 100ME-100BP fed broilers ($\log_2$FC = 6.11, Padj < 0.0001). All other genes showed nominal, non-significant expression differences. A few genes approached significance (Padj = 0.11), including IFT57 and NT5C1B, which were also upregulated in 100ME-85BP compared to 100ME-100BP fed broilers ($\log_2$FC = 1.09 and 0.51, respectively). Considering non-significant DEG with $|\log_2 FC| > 2.0$, expression of 41 genes was downregulated and 73 upregulated between 100ME-85BP and 100ME-100BP fed broilers. At 98% ME, 125 DEG were identified between 98ME-100BP and 98ME-85BP (Padj < 0.10). Six genes related to transcription and translation (ID2, RPS20, RPL23, RPL32, RPL35, RPL39L) and two involved in oxidative phosphorylation (CST3, ND3) were upregulated in 98ME-85BP compared to 98ME-100BP fed birds (Padj < 0.10). Balanced protein reduction also downregulated 51 genes involved in lipid/energy metabolism and 59 genes related to amino-acid/protein metabolism between broilers fed 98ME-85BP and 98ME-100BP (Padj < 0.10). Ongoing pathway analyses will further clarify the metabolic processes affected by graded dietary BP reduction. The present results point to a dietary energy-protein interaction, whereby reducing BP level slightly but significantly remodels small-intestinal gene expression only when ME is simultaneously reduced. None of the previously reported performance and carcass trait outcomes from this study showed such an interaction. Together, these findings may indicate that most of the jejunal metabolic pathways are preserved, but a modest adaptive intestinal response may occur to maintain alignment between nutrient absorption and metabolic demands under reduced dietary inputs. This may impact slow-growing broilers' nutrient excretions. Therefore, such insights are relevant when evaluating new nutritional strategies aiming to lower the environmental impact of slow-growing broiler production.

Untargeted metabolomics in urine and milk reveals coordinated nitrogen related metabolic responses to dietary protein degradability in dairy cows

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Untargeted metabolomics in urine and milk reveals coordinated nitrogen-related metabolic responses to dietary protein degradability in dairy cows Michela Errico1, Alessandro Catellani1, Federico Froldi1, Maurizio Moschini1, Antonio Gallo1, Gabriele Rocchetti1 1 Department of Animal Science, Food, and Nutrition (DIANA), Università Cattolica del Sacro Cuore, Via Emilia Parmense 84, 29122 Piacenza, Italy.Corresponding author: michela.errico@unicatt.it Optimizing nitrogen utilization in dairy cows requires identifying systemic metabolic responses to dietary protein degradability. We hypothesized that differences in rumen protein fractions would induce coordinated metabolic changes detectable within the low molecular weight range (50–250 m/z) in milk and urine. Nine dairy cows were assigned to a 3 × 3 Latin square design and fed three diets differing in protein composition: high soluble protein (SOL), high rumen-undegradable protein (BY), and a balanced control (CTR). After 21-day experimental periods, milk and urine samples were analyzed by UHPLC–HRMS Orbitrap. The UHPLC system consisted of a degassing system, a quaternary UHPLC pump, an autosampler device, and a thermostatically controlled Waters BEH C18 column (2.2 x 100 mm, 1.7 µm) held at 35°C. The mobile phase consisted of (A) water with 0.1% formic acid and (D) acetonitrile with 0.1% formic acid. The duration of the method was 11 min, the injection volume was 10 µL, and the flow rate 0.2 mL/min. The mass range in the full scan experiments was set at 50–250 m/z. Multiblock data integration (mixOmics) was applied to investigate shared metabolic signatures between biofluids. A strong correlation (69%) on the first predictive component (PC1) indicated coordinated systemic regulation between milk and urine. Urea emerged as a discriminant metabolite in milk under the SOL diet, reflecting enhanced nitrogen turnover associated with high soluble protein feeding. The first predictive component (PC1) was driven by metabolites related to nitrogen and intermediary metabolism, including urea, uracil, 6-hydroxynicotinate, N-acetyl-leucine, 5-methyl-cytosine, and organic acids such as malic and sebacic acid, indicating coordinated modulation of urea cycle activity, nucleotide metabolism, amino acid turnover, and tricarboxylic acid (TCA) cycle intermediates. Correlation network analysis further highlighted xanthurenic acid as a central hub linking tryptophan degradation to systemic nitrogen and energy metabolism. As a product of tryptophan degradation through the kynurenine pathway, it was strongly associated with urea, L-tryptophan, N-acetyl-leucine, and purine-related compounds, indicating tight coupling between aromatic amino acid catabolism and systemic nitrogen handling. Connections with citric acid and isocitrate further support integration between nitrogen disposal and energy metabolism. The involvement of ethanolamine and O-phosphoethanolamine suggests additional effects on membrane turnover and cellular remodeling. Overall, low-mass untargeted metabolomics effectively captured diet-specific metabolic signatures and revealed coordinated regulation of tryptophan degradation, urea cycle activity, and energy metabolism, providing mechanistic insight into nitrogen utilization dynamics in dairy cows.

Dietary vitamin D_3 dose-dependently modulates gene expression in peripheral blood mononuclear cells of dairy cows following an intramammary immune challenge

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While extensive research in dairy cow nutrition has allowed for a better characterization of dietary vitamin D3 (VitD) needs, especially in the context of calcium homeostasis, it is still unknown how these needs vary during periods of immune challenge (e.g., infections or the peripartum period). We evaluated transcriptional responses of peripheral blood mononuclear cells (PBMCs) in dairy cows following an intramammary lipopolysaccharide (IM-LPS) infusion under four VitD dietary supplementation levels (1X–4X baseline dose based on NRC requirements). Eight multiparous Holstein cows (90 JEL) from the CRSAD dairy herd were used in a duplicated 4 x 4 Latin Square design with 4 periods of 14 d each and 7 d washouts. Treatments were:1) 1X NRC requirements (Control; 1040 IU/kg DM); 2) 2X NRC requirements (2X; 2,080 IU/kg DM); 3) 3X NRC requirements (3X; 3,120 IU/kg DM) and 4) 4X NRC requirements (4,160 IU/kg DM).The IM-LPS challenge (25 µg of E. coli 0111:B4 LPS; EB Vaccigrade; InvivoGen) was performed at 8h00 on d12 of each period and blood samples collected immediately before and 4h after the challenge for PBMCs preparations. Total RNA was extracted from PBMCs (TRIzol) and quality assessed using an Agilent Bioanalyzer (median RIN = 4.7). To account for partially degraded samples, rRNA-depleted libraries were prepared and sequenced (PE 100 bp, Illumina NovaSeq). Libraries yielded between 49 and 72 million reads per sample. Reads were aligned to the bovine reference genome (NCBI ARS-UCD2.0) and differential expression analysis were performed using DESeq2 package (padj < 0.05, $|\log_2 FC| > 1.5$) and identified 90, 401, 350, and 267 significantly regulated genes in the 1X–4X comparisons (0h vs. 4h), respectively. Fifteen genes exhibited consistent differential expression status, which was also consistent with dose-dependent regulation across all treatments. Among these, PTX3 and CXCR6, key modulators of innate and adaptive immunity, were upregulated with increasing VitD, while DOK2, a negative regulator of T/NK signaling, decreased. These patterns suggest that VitD supplementation enhances immune responsiveness by promoting early inflammatory mediators and modulating inhibitory checkpoints. Our findings provide novel insights into the transcriptional mechanisms by which VitD modulates immune function, with potential implications for mastitis treatment and general immunomodulation during periods of stress in dairy cattle. Keywords: Vitamin D3, bovine mammary gland, RNA-seq, LPS, immune regulation, differential gene expression

Functional diversity of cannabinoid receptors in the Czech Red Pied and Czech Red cattle

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Although hemp (Cannabis sativa L.) plants were used as an effective fodder crop across Asia and Europe, their current use is restricted. The effect on the animals is considered potentially harmful in view of the psychoactive compounds present in a variety-specific manner. In cattle, the admitted addition to the fodder mixtures is not significant, according to a recently updated EFSA report from 2011. On the other hand, the susceptibility to the psychoactive cannabinoids is determined not only by the substance concentration, but also by the functional forms of cannabinoid receptors. Therefore, the diversity in the genes encoding cannabinoid receptors, CNR1 and CNR2), was investigated and the results were interpreted for local cattle breeds of the Czech Republic. The panel of breeds included Czech Red Pied cattle (CRP, Simmental type), the conserved nucleus herd of this breed, and the conserved Czech Red cattle (CR), which is considered an indigenous mountain breed. The pooled genomic DNA samples from 150 bulls of the CRP production population, 35 animals from the historical nucleus herd, and 80 animals of CR were sequenced using Illumina HiSeq technology. The obtained 60× coverage was sufficient to detect polymorphisms above the 0.05 frequency with 95% efficiency. The diversity as revealed against the UMD3.1.1 reference sequence was evaluated for each population. The gene CNR1 (located on chromosome 9, 29,042 bp) demonstrated 300 polymorphisms in commercial CRP, where 8 were located in mRNA (5616 nt) and 4 SNPs in the protein coding sequence (1419 nt). Nevertheless, only one SNP was associated with an amino-acid substitution (A>G at position 62659162, encoding the Met23Val). The variability in the nucleus herd of CRP was comparable, comprising 262 polymorphisms, 9 of which were located in mRNA and 5 in the coding sequence. In the CR population, 236 polymorphisms were found in the CNR1 gene, among them 9 in mRNA and 4 in CDS. The presence of an additional polymorphism (C>T at 62660127) in the coding sequence in the conserved nucleus herd of CRP confirms the previously suggested background of the historical cattle population. This picture was supported by the variability observed in the CNR2 gene (chromosome 2, 37,925 bp) that harboured 505 polymorphisms in the CRP population. Eight SNPs were in mRNA (3796 nt), and 3 SNPs in CDS of 1086 nt, where all SNPs were synonymous. A characteristic diversity can be assumed for the nucleus herd of CRP as based on the SNP 129632443A>G in CDS, which is absent in other populations. Statistical reconstruction of haplotypes suggests non-arbitrary grouping of SNPs, which might be ascribed to the historical origin or alternating selection pressure. A considerable number of polymorphisms found in coding and adjacent regions suggested variation in susceptibility to undesirable psychoactive compounds. The question arises as to what extent the variability in the cannabinoid receptor genes is caused by the selection pressure exerted by the use of hemp as feed in the pre-historical and medieval times. At the same time, a genetic estimate of cattle predisposition to the hemp feed might contribute to the use of the limits set by EFSA. At least a partial transition to hemp as a feed crop is supported by the presence of antimicrobial components and common reasons related to organic farming such as higher green matter productivity and soil recultivation.

Effect of dietary Lactobacillus Plantarum TWK10 on growth performance, nutrient digestibility, blood profile and gut microbiome in beagles

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Dogs have cohabited with humans for thousands of years and serve major companions. The health and well-being of dogs, like that of their owners, are closely linked to the composition and function of gut microbiota. Providing appropriate care and a nutritionally balanced diet is therefore essential for maintaining companion animal health. Therefore, this study aims to evaluate the effects of dietary supplementation with Lactobacillus plantarum TWK10 on the overall health of beagles. Eight healthy beagles with an initial body weight of 12.50 ± 1.28 kg were enrolled in a 10-week crossover study consisting of two experimental phases: Phase 1 (weeks 0–4) and Phase 2 (weeks 6–10), separated by a 2-week washout period. Dogs were assigned to receive either a control (CON) commercial diet or the CON diet supplemented with L. plantarum TWK10 at a dose of 1×10^{10} CFU/day. Growth performance, blood profiles, nutrient digestibility, fecal moisture and pH, and fecal microbiota and microbiome composition were assessed at the end of each phase. Data was analyzed using linear mixed models for growth performance, nutrient digestibility, fecal pH, and hematological parameters, with dietary treatment and experimental period as fixed effects and individual dogs as a random effect using SPSS version 22.0 (SPSS Inc., Chicago, IL, USA). Alpha-diversity indices were analyzed using the Kruskal-Wallis test, and beta-diversity was assessed by principal coordinate analysis using Bray–Curtis's dissimilarity and PERMANOVA. Our results showed that beagles fed Probiotic supplementation had no significant effects on growth performance, nutrient digestibility, or overall fecal microbiota composition; however, it resulted in reduced fecal pH and significantly increased serum calcium, insulin-like growth factor-1, creatinine, and immunoglobulin G concentrations compared with the CON group. While no differences were observed in alpha- or beta-diversity indices between groups. At the taxonomic level, the relative abundance of the phyla Firmicutes and Bacteroidota and the genus Prevotella was higher in the probiotic-treated group. Collectively, these findings suggest that dietary L. plantarum TWK10 (1×10^{10} CFU/day) may confer beneficial effects on gut health in beagles without altering production traits.

Integrative Genomic and Molecular Modeling Analyses Elucidate the Structural and Functional Impact of the GCKR P446L Variant in Type 2 Diabetes

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Type 2 diabetes mellitus (T2DM) is a complex metabolic disorder influenced by genetic and environmental factors. To identify functionally relevant variants associated with T2DM, we conducted a genome-wide association study (GWAS) in 35,120 individuals from the Korean Genome and Epidemiology Study (KoGES) meta-cohort, analyzing 465,971 genotyped single-nucleotide polymorphisms (SNPs). Among variants exceeding the genome-wide significance threshold ($P < 5 \times 10^{-8}$), the missense SNP rs1260326 (P446L) in the GCKR gene emerged as a genome-wide significant non-synonymous variant associated with T2DM. GCKR encodes glucokinase regulatory protein (GKRP), which modulates glucokinase (GCK) activity in hepatic glucose metabolism. To assess the functional consequences of the P446L substitution, structural modeling and protein–protein docking analyses were performed to compare interactions between GCK and GKRP in wild-type and mutant forms. The mutant protein exhibited enhanced binding affinity but altered interaction patterns within the GCK–GKRP complex, suggesting perturbation of normal regulatory dynamics. Molecular dynamics simulations further revealed reduced structural stability and altered conformational behavior in the mutant GKRP relative to the wild type. These structural alterations indicate that the P446L substitution may compromise GKRP-mediated regulation of GCK, potentially contributing to dysregulated glucose metabolism and increased T2DM susceptibility. By integrating large-scale population genomics with molecular structural analysis, this study provides functional evidence supporting the biological relevance of the GCKR P446L variant in T2DM.

Ensemble Stacking Model Based Integration of Omics Data Uncovers Dynamic Biological Mechanism Landscape and Prognostic Factors of Sleeve Gastrectomy in Morbid Obese

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Bariatric metabolic surgery achieves durable weight loss in morbid obesity, yet postoperative responses vary widely across individuals. Most prediction models rely primarily on clinical variables, limiting insight into molecular determinants of heterogeneous outcomes. We developed and internally validated a multi-omics stacking ensemble to predict 12-month percent excess BMI loss (%EBMIL) after vertical sleeve gastrectomy (VSG) using preoperative transcriptomic, metabolomic, and lipidomic profiles derived from subcutaneous adipose tissue (SAT), visceral adipose tissue (VAT), and blood. To improve learning stability in a limited-cohort setting, we performed data amplification (35×5) via smoothed bootstrapping. Base learners—including linear regression, ridge, lasso, elastic net, XGBoost, and random forest—were trained within each omics layer, and their predictions were integrated using an elastic net meta-model. We exhaustively evaluated tissue- and omics-layer combinations to identify the optimal predictive configuration. The best-performing ensemble combined blood lipidomics with SAT RNA-seq, SAT lipidomics, and VAT RNA-seq, achieving strong internal-validation performance (R^2=0.927521, MAE=2.604036, RMSE=3.274321). Pathway-based functional analysis of key predictive features highlighted features enriched in glycerophospholipid metabolism, implicating circulating glycerophospholipids as molecular signals associated with post-VSG lipid metabolic remodeling across interconnected organs and tissues, including liver, intestine, adipose tissue, and skeletal muscle. Collectively, this stacking-based multi-omics framework provides an accurate and biologically informed approach for predicting 12-month %EBMIL and prioritizing candidate biomarkers underlying inter-individual variability in surgical weight-loss outcomes.

The effect of reduced protein diet on epigenetic changes in muscles of Krškopolje pigs

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Krškopolje pig is a local breed with lower protein requirements compared to modern breeds. Biological mechanisms underlying this adaptation are poorly understood. It is hypothesized that diets with low protein (LP) content could lead to epigenetic modifications, influencing gene regulation in the muscle. It is assumed that such effects might differ in different production systems. The aim of the present study was to identify DNA methylation differences in the longissimus dorsi (LD) muscle of pigs fed a LP diet compared with animals receiving a standard high protein (HP) diet. Study was conducted in two production systems, organic outdoor and conventional indoor. In both systems, two experimental groups (LP, HP) were formed with 10 pigs per treatment (HP vs. LP). Pigs were slaughtered at 330 days of age and samples of LD muscle collected. DNA was extracted and analysed using GBS-MeDIP method. In organic outdoor production system, comparing LP and HP diet identified 16 differentially methylated regions (DMRs, $P<0.05$, FDR=0.439) located mainly in promotor and intronic regions. Twelve related-genes were associated with DMRs (e.g., MTOR, FGF9 and ZIC3), being significantly enriched in gene ontology terms such as muscle tissue development and tissue morphogenesis (Q-value < 0.05), as well as PIP3-activates-AKT signalling pathway (Q-value<0.01). In indoor conventional system, 12 DMRs were identified between HP and LP treatment ($P<0.05$; FDR=0.384) being mainly located in intergenic regions (75%). One DMR was located close to TKFC gene, which is involved in glycerol and carbohydrate metabolic process, and regulation of immune response. Since the effect of LP diet on epigenetic profile was small in both production system, we additionally assessed the effect of rearing system. We identified 55 DMRs ($P<0.05$; FDR=0.361), with related-genes enriched in endoplasmic reticulum subcompartment (OSBPL8, PEDS1, BCAP31), adherens junction (TRPV4, JUP), and apical plasma membrane (SLC5A10, TRPV4) cellular component terms (Q-value<0.05). Additionally, the RHO GTPase cycle pathway was significantly enriched (Q-value<0.05). In conclusion, the LP diet had a minor effect on DNA methylation consistent with lower protein requirements, while rearing system induced differences were more important. Acknowledgements: Funding by Slovenian Research and Innovation Agency (P4-0133, Z4-60178) and project GEroNIMO (EU H2020 GA no. 101000236) are acknowledged.

Maintaining agrobiodiversity: Holistic sensory and consumer testing of local chicken breeds and crossbreds thereof with commercial lines

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Crossbreeding with commercial lines presents an opportunity to improve the production performance of local chicken breeds and thus their economic viability while preserving agrobiodiversity through parental stocks. It remains to be shown whether sensory claims and information provision to consumers could support marketing efforts. Herein, a mixed method approach was applied to generate consumer insights and for objective sensory evaluation of three local breeds (Altsteirer, Ramelsloher, Bielefelder) and crossbreds thereof with commercial lines (White Rock, Ranger). Therefore, diverse culinary use cases as well as different marketing channels were considered for deriving implications for marketing strategies. For objective sensory assessment, chicken broths, sous-vide cooked breast meat, steamed meatballs, and fricassee, were evaluated under controlled conditions by trained sensory panelists via Quantitative Descriptive Analysis (QDA) and Napping. Various chefs were provided chickens and commented on their usage for higher level gastronomy. Additionally, a real-life consumer acceptance study (n = 642) was conducted in a local cafeteria whereby considering various dishes using breast and thigh meat; also an information intervention regarding the loss of agrobiodiversity was tested. Finally, through an in-home test (n = 108) consumers' willingness to use whole chickens for home cooking was tested. Overall, no or rather small sensory differences between local breeds and their crosses were found by trained assessors, with the differences being product- and preparation-specific. Broths showed strong variation in salty taste and fatty aftertaste ($p = .001$), favouring purebreds. In meatballs, differences were most apparent between breast and leg meat ($p < .048$) and sous-vide cooked breast samples showed textural differences ($p = .003$). The real-life cafeteria experiment revealed that overall liking was higher for commercial breeds. However, consumers preferred local crossbreed thighs over commercial breed thighs, namely with local breed thighs being more tender and less dry. Providing information about agrobiodiversity loss showed a significant increase of the overall liking of local breed dishes and improved consumer attitudes towards agrobiodiversity preservation. The home-use test revealed both opportunities and barriers for whole-animal utilization as the study revealed distinct segments varying in their attitudes. Importantly, the preparation of whole chickens is perceived too time-consuming (and demanding) by a relevant share of consumers, and also feelings of disgust were identified. As household income, family structure, cooking skills were found to influence both acceptance and willingness to pay, targeted approaches appear promising for marketing whole chickens. In conclusion, no clear sensory advantages of local breeds or their crossbreeds could be identified. In turn, their sensory properties don't hinder marketing, and the choice of crossbreeding partners had no substantial impact on the sensory qualities. The study highlights the potential of information dissemination to support local breeds' preservation. Future research should aim at validating consumer attitudes regarding the use of whole chickens as this is considered key for the marketing of local breeds (and crossbreds thereof). Finally, the sensory protocols developed herein add to the existing set of methods for a more holistic sensory analysis of poultry as well as for generating consumer insights with high external validity.

Assessment of post-thaw semen quality in two conserved Norwegian rooster lines

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The Norwegian gene bank for laying hens, established in 1974, maintains 12 heritage breeding lines at Hvam Upper Secondary School. Previous studies have shown that these lines are only distantly related to commercial layer populations. The gene bank is unique in its kind, and there is currently no backup of genetic material, neither as live animals nor as frozen germplasm. This highlights the need for a complementary ex situ cryopreservation strategy. To support this, the Norwegian Genetic Resource Centre conducted semen collection and cryopreservation trials in 2016 and 2018, resulting in 540 frozen semen doses. Despite subjectively assessed post-thaw motility appearing relatively high (≤ 40%), an insemination trial in 2018 resulted in extremely low fertilization rates (< 4%), prompting further investigation into post-thaw sperm quality. In this pilot study, we applied computer-assisted sperm analysis (CASA) to frozen-thawed semen from two conserved breeding lines, Jærhøns (JH) and NorBrid8 (NB8), to assess sperm motility impairment not detected by manual microscopy. Sperm motility was assessed from 24 individual doses (12 JH, 12 NB8), each representing one rooster and evaluated under two post-thaw storage methods, T1 and T2. In T1, motility was assessed immediately after thawing the straws in a 37 °C water bath for 30 seconds, whereas in T2 the remaining sample was held at 4 °C for up to 5 minutes before analysis to evaluate sensitivity to short-term cold exposure. Recorded traits included total motility (MOT), progressive motility (PROG), curvilinear velocity (VCL), average path velocity (VAP), straight-line velocity (VSL), straightness (STR), and linearity (LIN). To evaluate breed and storage method effects on sperm motility, we fitted linear mixed models with breed and storage method as fixed effects and rooster as a random effect to account for repeated measurements. MOT and PROG averaged 36.5% (SD 8.9%) and 19.9% (SD 5.9%), and both breed and storage method clearly influenced several CASA traits, with substantial variation attributable to individual males. NB8 exhibited lower MOT and PROG but higher VCL, VAP, and VSL than JH, while T2 reduced motility, all velocity traits, and lowered LIN without affecting STR. Principal Component Analysis (PCA) supported these patterns: PC1 (59% variance; velocity and linearity) and PC3 (12%; secondary kinematics) were influenced only by storage method, whereas breed differences were confined to PC2 (25%; motility). Correlation analyses further distinguished the breeds, with NB8 showing strong, coherent associations between motility and kinematic traits and JH displaying weaker or inconsistent covariance, indicating that NB8's motile sperm form a more integrated functional group despite lower MOT. This study represents our first step toward objectively characterizing sperm motility in frozen–thawed rooster semen, and future work combining similar analyses with controlled insemination trials will be essential to determine which sperm motility parameters—or composite indices—best predict fertilization success in Norwegian laying hen breeding lines.

Quo vadis Leicoma pig?

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The pig breed Leicoma was developed in the 1970s in the former German Democratic Republic as a synthetic maternal line and officially recognized in 1986. Its genetic foundation comprises German Landrace, Dutch Landrace, and Duroc, with minor contributions from Estonian Bacon and German Sattelschwein. Following German reunification and the structural transformation of the pig industry, the population declined drastically, and the breed has been classified as endangered since 2010. A severe genetic bottleneck in 2013–2014 reduced the effective breeding population to one boar and 19 sows. To counteract the loss of genetic variability, five historical sire lines were reactivated in 2016 using cryopreserved semen from the national in vitro gene bank. The objective of this study was to evaluate the effectiveness of these conservation measures by jointly examining reproductive performance and population genetic parameters under practical breeding conditions. The dataset comprised 1,158 litters from 317 herdbook sows recorded between 2019 and 2025 on a conservation-oriented farm in Saxony-Anhalt. The data was provided by the supervising pig Breeding Association Baden-Württemberg, known as German Genetics. The traits analyzed were number of piglets born alive (NBA) and number of piglets weaned (NW). Further traits can be found in the abstract by Frenkel et al. (2026, abstract # 1252314348).To account for physiological differences between parities, first parity (n = 317) and later parities (n = 841) were analyzed separately. Models were develeoped based on univariate linear models in SAS. The statistical models finally included year-season and breed of the mating partner as fixed effects. For later parities, parity number was fitted as an additional fixed effect. Age at first farrowing (first parity) or farrowing interval (later parities) was included as covariate. Random additive genetic effects were modeled using the numerator relationship matrix derived from the complete pedigree (N=647) in BLUPF90 under an animal model. Variance components were estimated by restricted maximum likelihood (REML), and heritabilities were calculated as the ratio of additive genetic variance to total phenotypic variance. Individual inbreeding coefficients were computed from pedigree information. In the pure line, mean NBA was 10.3 in first parity and 10.5 in later parities, while mean NW was 8.4 and 9.1, respectively. Heritability estimates for NW and NBA were between 0.09 and 0.25. Despite the small sample size, these values for fertility traits are within the expected range. The results correspond to the breeding goals from the 1990s, when NBA was to be achieved at 11.5 and NW at 10.3 piglets per sow and year. The moderate heritabilities indicate that additive genetic variation has been maintained despite the recent bottleneck, allowing continued genetic improvement within a conservation framework. Comparison with historical data (1973–1996) demonstrated stable fertility performance and no indication of inbreeding depression. The maximum inbreeding coefficient observed was 0.30, whereas the mean coefficient amounted to 0.06 among inbred animals and 0.04 across the total population. Given the bottleneck experienced a decade earlier, these values suggest effective management of relatedness and successful reintroduction of genetic diversity through cryoconserved material. Molecular genetic analyses of piglets born in 2025 were additionally conducted by bloodline as given by the pedigree to verify the effective contribution of the reactivated sire lines and to further characterize current genetic variability.

Genomic relationships among four Northwestern European dual-purpose cattle breeds: Implications for strategic exchange and preservation of unique and shared genetic diversity.

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Dual-purpose black and white cattle were the most common type of cattle in Northwestern European countries until the late 19th century, valued for their high milk production and decent slaughter value. Nowadays, they are largely replaced by Holstein Friesian cattle, a specialized dairy breed selected for high-input farming systems. However, increasing interest in agroecological and organic farming systems provides new opportunities for the original dual-purpose breeds. These cattle are well-suited for extensive low input systems which require robust, self-sufficient, healthy cows with high fertility. Even though interest in local dual-purpose cattle breeds has increased, their economic gain in conventional agriculture is limited, and consequently most of these breeds are rare. Because of common origin and overlapping breeding goals, these local dual-purpose cattle breeds might benefit from strategic exchange of breeding animals. More specifically, exchange of genetic material of breeding bulls between (commercial) artificial insemination (AI) centres or national gene banks may be promising. Here, we examine genomic diversity within and between four dual-purpose cattle breeds from the Netherlands (Dutch Friesian), Ireland (Irish Pure Friesian), United Kingdom (British Friesian) and Germany (Deutsches Schwarzbunte Niederungsrind), and compare to Dutch Holstein. We combined SNP datasets with 100-300 individuals per breed, generated with different genotyping methods, and filtered down to 20K SNPs with <10% missingness in the combined dataset. We find considerable overlap between the dual-purpose breeds in PCA and STRUCTURE plots, reflecting the common ancestry and continued exchange of individuals. But each local breed also harbours unique variation, in line with slight differences in breeding goal, adaptation to local circumstances, and random genetic drift. Holstein show as completely differentiated from all four dual-purpose breeds and not suitable for (regular) exchange of breeding animals. Exchange between the dual-purpose breeds requires a balance between bringing in genetically dissimilar breeding stock, and staying true to the individual breed characteristics. Simulations show that (limited) exchange of available breeding material via AI centres and national gene banks can contribute to overall conservation of genetic diversity for all four breeds while preserving unique variation. Furthermore, collections in gene banks are built up over time, aiming to include as much of the genetic variation present in the in situ population as possible. This long-term collection presents a wide variety of genetics available for exchange between breeds, allowing for a more strategic approach using a combination of earlier and modern genetics of each breed. We show an approach of exchanging frozen semen with a limited number of matings per bull between all breeds to be cost-effective and most successful conserving genetic diversity overall and within breeds.

Genomic analysis of genetic diversity and population stratification in Swabian-Hall pigs

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Understanding genetic structure and levels of inbreeding within and between farms is essential for sustainable breeding management, especially in small endangered local breeds. Subdivision among populations contributes to genetic differentiation, with within-farm mating practices influencing the distribution of heterozygosity and inbreeding. Therefore, genomic selection has become standard practice to monitor breeds and guide breeding programs. In this study, SNP array genotype data were analyzed to assess the genetic structure, differentiation, and inbreeding of Swabian-Hall pigs from three farms P, E and W. To ensure data quality, SNPs with MAF < 0.05, markers with >5% missing genotypes, and individuals with >5% missing data were removed. LD pruning was then applied to marker independence. Principal component analysis (PCA) was performed to visualize population structure, and Wright's F statistics were calculated to quantify genetic differentiation and inbreeding. ADMIXTURE analysis was conducted for K = 2–4 to examine the genetic composition of individuals. The PCA reveals that individuals from the farms W and E were relatively close to each other, indicating a high degree of genetic similarity, whereas farm P shows a slight shift along the first principal component, suggesting moderate differentiation. The pairwise FST values support this pattern as differentiation between W and E is lowest (0.0259), while the comparisons between P and W (0.0588) and P and E (0.0548) show higher differentiation. This confirms that W and E are genetically more closely related to each other than to P. The total FST value of 0.0479 indicates low to moderate genetic differentiation overall. Negative FIS values in all farms indicate a slight excess of observed heterozygosity compared to the Hardy-Weinberg expectation. The following ADMIXTURE analysis shows similar genetic makeup. Individuals from W and E exhibited comparable cluster proportions, while individuals from P showed partially different proportions. Together, PCA, FST, and ADMIXTURE consistently indicate that farm P is genetically more distinct, whereas W and E are more similar. The genomic analyses provide important insights for improving conservation strategies within the breed. By integrating PCA, FST, and ADMIXTURE results, subtle population structure and potential inbreeding risks can be identified. This information allows informed mating and animal exchange decisions that maintain genetic diversity while supporting performance traits. In this study, the observed stratification between farms could partially result from differences in blood refreshments and crossbreeding history, leading to varying allele frequencies. To reduce stratification, greater animal exchange between farms, particularly between P and W/E, is recommended. However, in order to improve conservation and breeding strategies within the breed, it is sometimes essential to carefully weigh performance characteristics against the preservation of genetic diversity. Further research is needed to refine these strategies and provide more information on optimizing the breeding program for Swabian-Hall pigs.

Genetic Insights into the Diversity and Structure of European Draft Horse Breeds

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For centuries, draft horses have been an essential component of agriculture, forestry, and transportation, contributing substantially to the economic development of European regions while also shaping the cultural landscape and traditional farming systems. The aim of this study was to evaluate genetic diversity, population structure among European draft horse breeds. We analysed 1,042 individuals from twelve breeds – Belgian, Clydesdale, Czech Moravian Belgian, Franches-Montagnes, Muraközi/Međimurje horse, Noriker (Austria), Noriker (Czechia), Noric of Muran, North Swedish Horse, Percheron, Shire, and Silesian Noriker – using a dataset containing a minimum of 50,042 SNPs per individual. Observed heterozygosity was comparable across breeds (approximately 0.26), with the exception of Noriker (Austria) (Ho=0.34). Genomic inbreeding coefficients estimated using runs of homozygosity (FROH) and homozygosity-by-descent segments (FHBD) were generally moderate, ranging from approximately 0.03 to 0.14 (FROH>2Mb) and from approximately 0.05 to 0.14 (FHBD), whereas Clydesdale showed higher values compared to others (FROH>2Mb=0.26; FHBD=0.22). In contrast, most FIS values were negative, indicating preferential mating among unrelated individuals. Estimates of contemporary effective population size ranged from approximately 66 to 236 individuals across breeds. Estimated historical effective population size detected a demographic decline in most breeds between 25 and 8 generations ago. These results provide important insights into the current levels of genetic diversity and demographic history of European draft horse breeds and may support future conservation and breeding strategies. This work was supported by projects GACR 24-14325L, QL25020022, APVV-20-0161, VEGA 1/0316/25 ,IP-2022-10-8926, and the Flagship Research Groups Programme of the Hungarian University of Agriculture and Life Sciences.

Pedigree analysis and genomic characterization of two local German cattle breeds

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Local dairy cattle breeds represent a unique genetic and cultural heritage and should be conserved for future generations. In addition, these breeds are well suited to a wide range of different environments and may play an important role in adapting breeding programs to future production challenges. The aim of the present study was to estimate pedigree- and genome-based inbreeding measures, the number of effective founders, and marginal genetic contributions of key ancestors in two Northern German cattle breeds (Angler dairy cattle (ADC) and Red and White dual purpose cattle (RDN)). Pedigree and SNP-chip data were provided by Vereinigte Informationssysteme Tierhaltung w.V. The pedigree file for the ADC breed contains information on 132,611 animals born between 1906 and 2025, while the RDN pedigree includes 155,813 animals born in the same period. SNP data were available for 7,570 ADC and 5,318 RDN animals, respectively. In the ADC pedigree, 72,887 animals were inbred with an average inbreeding coefficient (F) of 0.024 and a maximum F of 0.380. In the RDN pedigree, 45,822 animals were inbred with an average F of 0.026 and a maximum F of 0.315. To estimate the effective number of founders and marginal genetic contributions by breed and sex, three reference populations were defined. Reference population 1 (Ref1) included animals born between 2010 and 2014, Ref2 included animals born between 2015 and 2019, and Ref3 comprised animals born between 2020 and 2025. The number of bulls in Ref1, Ref2, and Ref3 for ADC was 1,720, 1,237, and 1,507, respectively, whereas in the RDN population the numbers were 894, 571, and 1,361. For cows, the corresponding numbers were 7,610, 9,471, and 14,539 in ADC and 5,746, 5,096, and 7,168 in RDN. For ADC bulls, the number of effective founders decreased from 424.2 in Ref1 to 344.5 in Ref3. In the RDN breed, the number of effective founders for bulls declined from 250.9 in Ref1 to 154.7 in Ref3. For ADC cows, the effective number of founders ranged between 302.5 (Ref2) and 312.6 (Ref3), whereas in the RDN breed it ranged between 154.4 (Ref3) and 173.3 (Ref2). In the RDN breed, the highest marginal contribution of a single animal to the reference population was 0.07, 0.06, and 0.07 for Ref1, Ref2, and Ref3, respectively, which was higher compared to ADC (0.04, 0.04, and 0.05). In ADC, the marginal genetic contributions of the 20 most influential animals ranged from 0.36 (cows in Ref1) to 0.58 (cows in Ref3). In the RDN breed, the corresponding values ranged from 0.48 (cows in Ref1 and bulls in Ref1) to 0.55 (cows in Ref3). Genomic characterization was performed using a subset of 1,901 ADC animals. After filtering the genotype data for minor allele frequency < 0.01 and SNP call rate < 0.99, 40,405 autosomal SNPs remained for estimating genome-based inbreeding coefficients using the software Plink. Inbreeding coefficients based on runs of homozygosity (ROH) were calculated using two different parameter settings. The first parameter set detected 24,560 ROH and the estimated FROH varied between 0.002 and 0.288 with a mean of 0.043. In the second parameter set, FROH varied between 0.001 and 0.284 and the number of detected ROHs was 21,519. Genomic characterization will be extended to all ADC and RDN animals. Additional genomic inbreeding coefficients will be estimated from the diagonal elements of the genomic relationship matrix, and ROH islands will be identified in both breeds.

Historical expansion and severe recent demographic contraction in the South African Beef Shorthorn population

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The South African (SA) Shorthorn is one of the country's oldest beef breeds and has historically contributed genetically to several composite beef breeds, globally and locally, including the SA Bonsmara and SA Beefmaster. However, despite its historical importance, the breed has undergone a substantial demographic decline, now represented by only ~13 active breeders, coinciding with the expansion of composite breeds developed using Shorthorn genetics. This study aimed to characterise the demographic trajectory and genetic diversity of the SA Shorthorn population using pedigree records. Pedigree data comprising 35 444 animals born between 1925 and 2024 were analysed. Pedigree depth was moderate, with a mean of 2.71 complete generation equivalents (CGE) and a maximum of 9.18 generations, while the mean generation interval was estimated at 5.16 years. A total of 4 389 founders were identified, representing 12.4% of animals in the pedigree, although unequal founder contributions resulted in an effective number of founders (f_e) of 232.7. The effective population size (N_e), estimated from the rate of inbreeding, was 103.97. Demographic analysis revealed a pronounced contraction in census size, with registrations peaking at 1 919 animals in 1989, followed by an 86.9% decline in the contemporary population. A reference population of animals born ≥2015 (n = 2 510) was defined to represent the modern cohort. Within this population, 127 sires contributed progeny with a mean of 19 offspring per sire, and a Gini coefficient of 0.53 indicated moderate reproductive inequality among males. These results demonstrate that although the SA Shorthorn originated from a relatively broad founder base, the contemporary population has undergone substantial demographic contraction. Monitoring demographic trends and genetic diversity will therefore be important to support the sustainable management and conservation of this historically important breed.

Genomic Structure of Local Sheep Breeds in Tunisia Using SNP Markers

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Tunisian sheep breeds represent important genetic resources adapted to diverse environmental conditions, yet their genomic structure and diversity patterns remain insufficiently characterized. In this study, genome-wide SNP data from 180 individuals belonging to four Tunisian breeds (Barbarine, Noir de Thibar, Queue Fine de l'Ouest, and Sicilo-Sarde) were analyzed to investigate genetic diversity, population structure, differentiation, and demographic history. Following stringent quality control filtering, genetic diversity indices (observed and expected heterozygosity and FIS) indicated moderate within-breed variation and low levels of inbreeding across populations. Pairwise FST estimates revealed low to moderate genetic differentiation (0.004-0.029), with Sicilo-Sarde emerging as the most distinct breed. Multivariate analysis (DAPC) and model-based clustering (LEA) consistently identified clear genetic separation of Sicilo-Sarde, while Noir de Thibar exhibited higher admixture signals, suggesting historical gene flow among Tunisian breeds. Linkage disequilibrium decay patterns differed among breeds, with slower LD decay observed in Noir de Thibar, indicating potential differences in effective population size or demographic history. Runs of homozygosity analyses revealed generally low genomic inbreeding (FROH), although increased variability was detected in Queue Fine de l'Ouest. Phylogenetic reconstruction based on Nei's genetic distance supported the differentiation patterns observed in structure analyses. Overall, these results demonstrate moderate population structure among Tunisian sheep breeds, highlight the genetic distinctiveness of Sicilo Sarde, and provide valuable insights for the conservation and sustainable management of local ovine genetic resources.

Genomic effective population size in two lines of mice divergently selected for homogeneity of birth weight

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Effective population size (Ne) is a key indicator of genetic diversity and a fundamental parameter for monitoring extinction risk in populations. Traditionally, Ne has been estimated using pedigree information; however, decreasing genotyping costs have greatly expanded the availability of genome-wide data, enabling more detailed and temporally resolved inference. As part of a divergent selection experiment for environmental variability of birth weight, two mouse lines were developed: a high variability line (HL) and a low variability line (LL). Previous results indicated that LL outperformed HL in terms of production, animal welfare, and robustness. The aim of this study was to estimate Ne using genomic-based methods in order to evaluate the impact of sustained selection and to compare both lines. A total of 1,824 individuals from 26 generations were genotyped using the Affymetrix Mouse Diversity Genotyping Array (616,137 SNPs). For the present analysis, 35 HL and 41 LL individuals from generation 26 were considered. After quality control, 217,770 SNPs were retained. Effective population size was estimated using the linkage disequilibrium (LD) method, across the full spectrum of recombination distances between SNPs, allowing inference of both contemporary and historical Ne. Contemporary Ne was estimated using NeEstimator and currentNe, whereas historical Ne trajectories were inferred using GONE2. The results revealed a pronounced decline in Ne approximately 80-100 generations ago, consistent with the origin of the experimental population. Around 150 generations in the past, Ne exceeded 1,000 individuals in both lines, with consistently larger values in LL. This divergence likely reflects the long-term effects of the divergent selection regime, suggesting higher genetic variability compared to HL despite similar selection intensity and mating systems. Contemporary Ne estimates were below 50 in both lines, with only minor differences between them. Estimates obtained with NeEstimator (18.6 for LL and 20.2 for HL) were lower than those from currentNe (37.79 for LL and 41.80 for HL), likely due to methodological differences in the treatment of linkage disequilibrium. While NeEstimator assumes independent loci and relies on unlinked SNPs, currentNe accounts for recombination and physical linkage, enabling the use of all markers and generally producing higher estimates. Overall, genomic-based approaches provided results consistent with the known demographic history and selection design of the population, while offering improved temporal resolution compared to pedigree-based estimates. These findings highlight the value of genomic methods for disentangling recent and historical changes in effective population size under long-term selection.

Preliminary analysis of ancestral genomic effective population size in a SRC Iberian pig population selected for homogeneity of birth weight

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In recent decades, interest has increased in promoting homogeneity by reducing variability in traits. This trend is largely driven by economic incentives, as farmers receive higher inputs when defined production standards are achieved. In addition, selection for uniformity has been associated with improved robustness and resilience in several species. Since 2022, a selection experiment aimed at increasing birth weight homogeneity has been conducted in an Iberian pig population from Sánchez Romero Carvajal (SRC) fitting discrete generations. The study aimed to estimate genomic effective population size (Ne) in two generations of the selected SRC Iberian pig population. A total of 95 individuals corresponding to 38 trios from the first two generations were genotyped using the Axiom PorcineHD v1 array (Affymetrix). After quality control, unmapped SNPs, SNPs located on sex chromosomes, and SNPs showing Mendelian errors were removed. A total of 550,896 SNPs were retained for the analysis. The pedigree included 1609 individuals with a mean of 4.61 equivalent generations. Ancestral Ne was estimated using the linkage disequilibrium method, assuming population structure (Ne_{ax}) and not assuming structure (Ne_a). Moreover, Ne was calculated from pedigree information using the individual increase in inbreeding (Nei). GoNe2 was used to estimate Ne_a and Neax and ENDOG v4.9 to calculate Nei. The Nei was 47.54. This was consistent with the mating strategy implemented, in which 14 males from the best 14 dams were mated to 154 females from the best 52 dams per replicate performing two replicates per generation, and the mating avoiding the sharing of grandparents. Ne_{ax} was 26.98 one generation ago and gradually increased to 109.41 approximately 150 generations in the past. Ne_a ranged from 25.45 one generation ago to 45.43 thirteen generations ago and then increased from 131.40 fourteen generations ago to 790 approximately 150 generations ago. Ne_a may reflect formation of the SRC main herd in the 1980s (approximately seven generations ago), showing a sharp decline in Ne around thirteen generations ago. In contrast, Ne_{ax} showed a more gradual decrease in Ne over time. However, a small decline observed five generations ago (Ne_{ax} = 21.7) could also be associated with the formation of the SRC herd. Ne_{ax} detected two subpopulations within the analyzed population. The identified structure could be explained by the fact that this is a selected population, which could carry a family-based structure despite only two generations of selection are analyzed here. In addition, these animals were selected from a population based on the number of live piglets born and the total weight of the litter at 21 days, as well as for carcass quality traits. Nevertheless, Ne was also consistent with the historically demographic changes that occurred in this population. This preliminary study suggests that Nei captures current genetic diversity and mating structure in the SRC population selected for birth weight homogeneity. Furthermore, the present analysis also suggested that approaches that infer ancestral effective population size from genomic data successfully detect historical demographic changes in selected animal populations. Grant: PID2023-149012OB-I00 (RoPig).

Preserving genetic diversity in the local Belgian Piétrain pig populations.

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The local Belgian Piétrain pig breed originated in the village of Piétrain in Wallonia (South of Belgium) in the early 20th century, through targeted selection and probable historical introgression with Berkshire into the local white pig population present at that time. The breed is typically characterized by its extreme muscularity, lean carcass composition, and characteristic white coat with black spots. Since then, it has been widely used as terminal sire in crossbreeding programs worldwide to improve carcass quality of the offspring. The Belgian Piétrain therefore demonstrates how the unique genetic and phenotypic traits of a local breed can generate significant value nowadays for regional and international livestock systems. Preserving genetic diversity of this local population inside the global Piétrain gene pool is essential for long-term resilience, prevention of inbreeding depression, and future breeding innovation, and therefore requires close monitoring. So far, the conservation of the original populations of the Piétrain in Belgium has relied on a network of independent breeders rather than large breeding companies, which means each breeder preserves a part of the breed's global genetic diversity, as shown in a recent pedigree- and phenotype-based study. However, in recent years, the Belgian Piétrain population has been declining, and has even been characterized since 2024 as an endangered breed. This threatens the continuity of these local populations and their genetic diversity, which has shown its importance on a much larger scale. Therefore, the goal of this study is to provide insights into the genetic diversity of the Walloon Piétrain population by analyzing SNP genotypes obtained from the routine genetic evaluation. We will use 185 purebred progeny-tested Piétrain boars, genotyped between 2019 and 2024 for genomic characterization of the population. Genomic relationships stratified among breeders will be determined to identify intrinsic particularities of the Walloon Piétrain population. We will estimate the average level of inbreeding based on runs of homozygosity (ROH), and the effective population size. Furthermore, breed-substructure will be analyzed using principal component analysis (PCA). This genomic characterization will complement the previous pedigree- and phenotype-based study, supporting the valorization and sustainable management of the local Piétrain breed. This will benefit the regional breeding context while also positioning the population as a gene reserve for the global pig industry.

Leicoma Pigs – a Traditonal Breed Revived

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The Leicoma pig breed was developed in the 1970s in the former German Democratic Republic (GDR) as a synthetic breed functioning as the maternal line within a hybrid breeding program. In the creation of this synthetic line, genetic material was drawn from the following breeds: German Saddleback, Dutch Landrace, Estonian Bacon Breed, German Landrace, and Duroc. In 1985, the Leicoma accounted for approximately 25 % of the pig population in the GDR. Following German reunification, the breed experienced a severe decline in numbers. Although the use of cryopreserved semen from original Leicoma boars temporarily stabilized the population and even increased its genetic variability, the breed since the end of the Nineties has been classified as an endangered conservation population and is listed on the Red List of endangered livestock breeds. In light of this development, the present study aimed to evaluate the performance of the current Leicoma population. Firstly, performance traits were examine in relation to the breeding objectives defined in 1986 . Secondly, these results were compared with historical performance data from original Leicoma populations collected between 1973 and 1996. This approach allowed conclusions to be drawn regarding the current status of the breed following the population bottleneck observed with one boar in 2013 and 19 sows in 2014, while in 2024, 11 boars and 370 sows resulted in an effective population size of 42.7.The analysis was based on two datasets from one farm provided by the Pig Breeders´ Association Baden-Württemberg, known as German Genetics. Data contained information on reproductive traits (based on 1,158 litters from 317 sows, Schafberg et al. (2026, abstract # 1252314341)), fattening, and slaughter performance of the present Leicoma population (29 castrated test animals, performance testing in Ruhlsdorf during individual periods from 11/2022 to 8/2023). Although the breeding goals defined in the 1980s have not yet been fully achieved, the results indicate that performance in all three performance areas of the current population has remained almost unchanged from its best times. The following means (standard deviations/breeding goal) were observed: average daily gain 896 g (77.7/950), lean meat percentage in percentage 49.4 (3.1/53), carcass length 107.5 cm (2.4/100), pH45 of the loin 6.3 (0.2/6.4). An inbreeding depression resulting from a potential increase of homozygous genotypes could neither be confirmed nor ruled out. The particularly small effective population size and the associated increase in the average inbreeding rate per generation suggest a deviation from optimal breeding conditions. Likewise, the results do not provide evidence of shift toward any of the founder breeds. Further clarification would require at least a SNP genotyping of the current population which is actually in progress. Considering the observed positive performance trends and recent successful examples of marketing Leicoma as a niche product, the Leicoma pig can be regarded as being on a promising path toward revitalization. Nevertheless, the low effective population size continues to indicate an endangered status. In accordance with the Convention on Biological Diversity (1993), the Leicoma breed represents an important animal genetic resource contributing to biodiversity and should be strengthened with respect to both population size and genetic diversity while preserving breed-specific characteristics.

From genes to practice: breeding for intestinal health and resilience

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Abstract: Post-weaning diarrhea (PWD) remains one of the most prevalent and economically significant health challenges in pig production, leading to increased morbidity, reduced growth performance, higher antimicrobial usage, and considerable financial losses. The continued rise in litter sizes has resulted in more piglets with reduced robustness, further exacerbating the incidence and impacts of PWD. To uncover the intrinsic mechanisms that shape early-life robustness in piglets and to identify key factors influencing PWD and subsequent antibiotic use, we conducted an extensive clinical characterization of 2,500 pigs with a strong emphasis on gut health—a central determinant of overall resilience. Through genome-wide association analyses, we identified genomic regions and variants linked to health and robustness indicators. These insights provide a foundation for integrating health- and resilience-related traits into future breeding strategies, supporting more sustainable pig production and improved porcine health management.

A dual-challenge model simulating subclinical post weaning enteric dysfunction in piglets: Impacts on gut integrity and markers of inflammation

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There is an urgent need for robust experimental animal models that reliably mimic post-weaning enteric disturbances to support development of effective nutritional and management strategies for early-life pig health. A novel dual-challenge model was developed to induce a subclinical enteric disturbance by combining chemical stimulation with dextran sodium sulfate (DSS), which provokes mucosal disruption and compromises epithelial integrity, and bacterial stimulation using Escherichia coli, a common post-weaning enteric pathogen. It was hypothesized that such approach can provide a physiologically relevant simulation of early post-weaning intestinal dysfunction, offering a valuable tool for investigating how nutritional interventions influence resilience, gut function, growth performance, and the potential to reduce antibiotic use, while also allowing assessment of the role of pre-existing intestinal inflammation in shaping subsequent pathogenic infection. A total of 24 piglets (26 days old; 6.5 ± 1.7 kg BW; mixed sex; Landrace × Yorkshire × Duroc) were included in the study. The piglets originated from six sows that were homozygous positive for ETEC F4 susceptibility. The study included four treatments, n=6 piglets assigned to each: DSS, E. coli, a combined DSS + E. coli challenge, and a negative control. After a 7-day adaptation period, piglets assigned to the DSS treatments received 0.32 g DSS/kg BW dissolved in sterile saline with apple juice, while the remaining groups received a sham solution. On day 3, piglets in the E. coli groups were orally inoculated with E. coli O149:F4 (strain 9910045-1 ETEC), followed by once-daily reinoculation for two additional days. In vivo blood plasma samples were collected for sodium and haptoglobin analyses and fecal samples to assess pH and dry matter content, while intestinal tissues were collected post-mortem for tight junction gene expression and mucus cytokine analysis. RNA was extracted (NucleoSpin Tissue RNA Plus), reverse-transcribed, and analyzed by qPCR using TaqMan chemistry. Cytokines were quantified via a Porcine Multiplex Kit (R&D Systems) on the MAGPIX® platform. Data were analyzed by linear mixed models. Both DSS ($P = 0.005$) and E. coli ($P=0.02$) challenges, decreased faecal dry matter and, in the case of DSS, faecal pH ($P=0.01$), confirming that the model effectively induced gastrointestinal disturbances. Plasma sodium concentration increased with progression of DSS administration followed by E. coli challenge ($P=0.003$), implying potentially compromised intestinal absorption. Additionally, haptoglobin, a marker of systemic inflammation, showed markedly elevated concentrations following the E. coli challenge ($P=0.0004$), indicating a pronounced acute-phase response in the challenged piglets. Challenged groups showed a significant increase in MUC2 expression ($P<0.001$), reflecting promotion of mucus-mediated protective mechanisms. Importantly, the typical DSS-induced histological lesions, such as ulceration and substantial epithelial ruptures, were absent, suggesting mild-grade mucosal disturbances without severe tissue damage. Hence, the model successfully induced a mild-grade enteric inflammation characterized by subtle physiological and mucosal alterations without causing severe tissue damage or clinical disease. These results provide a reliable mechanistic platform for future studies aiming at evaluating nutritional, and non-antibiotic strategies to enhance post-weaning gut resilience and reduce antimicrobial use during the weaning transition. The study was conducted in the PIG-PARADIGM project funded by the Novo Nordisk Foundation under the grant number NNFSA210073688.

Blood Transcriptomic Determinants of Post-Weaning Diarrhea in Commercial Pigs

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In pig production systems, post-weaning diarrhea (PWD) is a significant challenge, resulting in impaired growth, increased morbidity, diminished animal welfare, and substantial economic losses. Because PWD is a major driver of antimicrobial use, it contributes significantly to the development of antimicrobial resistance. Identifying early-life molecular signatures that predispose pigs to PWD could enable biomarker-based risk prediction and support targeted antibiotic-free treatment strategies. In this study, we worked with pigs kept in a conventional production system. The pigs were treated individually, and no batch medication was allowed. We performed whole-blood mRNA sequencing from samples obtained the day before weaning (day 25) to identify transcriptomic differences between pigs that remained healthy or experienced self-resolving diarrhea, and those that developed clinically relevant PWD requiring antimicrobial treatment during the 10 days following sampling. From 243 pigs, we identified 16 that required PWD treatment in this period and randomly selected 16 healthy controls. Differential expression analysis (PWD-treated vs. Healthy), controlling for sex and pen, revealed 101 differentially expressed genes ($|FC| > 1.5$, q-value < 0.05). Healthy pigs showed upregulation of genes involved in amino acid homeostasis and growth efficiency, i.e., RRAGD and SH3BP4, suggesting more efficient nutrient sensing and regulatory control of mTOR-related pathways, mechanisms that may help immune cells adapt to dietary change and weaning-associated stress. The healthy group also showed upregulation of immune-modulatory genes, including CCR4, CD163L1, and TNFSF11, consistent with an active but non-inflamed immune state. In contrast, PWD-treated pigs showed a strong activation of cytotoxic and natural killer-associated pathways, including KLRK1, an activating receptor that responds to stress-induced ligands to drive NK- and T-cell cytotoxicity. Upregulation of Th1-related genes (TBX21, IL12RB2, IL2RB, and CD244) indicated a shift toward a cell-mediated, inflammatory immune response in the PWD-treated group. This was accompanied by elevated chemotactic and trafficking signals (CCL5, S1PR5, CX3CR1), suggesting active mobilization of immune cells to tissues. Finally, upregulation of genes involved in cell death and compensatory anti-inflammatory mechanisms, such as FASLG and IL10, are consistent with an immune system already responding to stress in these pigs before weaning. Together, these results suggest that PWD-treated pigs enter the post-weaning period already displaying increased cytotoxic and inflammatory immune activation, whereas resilient pigs exhibit metabolic robustness and a well-regulated immune status. Future work includes: 1) examining the blood transcriptome of these pigs at later stages of life and 2) integrating additional -omic layers (e.g., gut microbiome, genomic data) to better characterize how the pig immune system response evolves under different pathogen or management stressors. Such an integrative approach may clarify which biological pathways are consistently associated with resilience or susceptibility to PWD and support the identification of molecular signatures that could contribute to the development of practical weaning-risk assessment tools in pigs. The research was conducted in the PIG-PARADIGM project, funded by the Novo Nordisk Foundation under the grant number NNFSA210073688.

Digestibility and fibre degradation of wheat bran and Palmaria palmata in post-weaning piglets

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Seaweeds have attracted significant attention in recent years as potential feeds or feed additives, since their cultivation is not associated with use of arable land suitable for human food production. Most seaweed species have a low digestibility due to the unique and complex carbohydrate (CHO) structure of cell walls and storage polysaccharides. However, Palmaria palmata was found to be quite different from other red seaweed species because its fibres are resembling arabinoxylans from terrestrial plants. The objective of this study was to evaluate the potential of the red seaweed P. palmata as a functional feed ingredient for post-weaning piglets, with emphasis on fibre digestibility and degradation. We hypothesised that the higher content of soluble fibre, primarily mixed β(1→4, 1→3) xylans from P. palmata, would increase digesta viscosity but enhance fibre fermentation. A total of 32 piglets (initial body weight 6.21 ± 1.15 kg) were weaned at 28 days of age and assigned to two dietary treatments for 21 days in two blocks of 16 piglets. Piglets were housed in pairs from the same sow per pen. Diets were based on brown rice and fish meal to ensure that dietary fibre originated predominantly from added P. palmata (PP) or wheat bran (WB). Feed and water were provided ad libitum. All piglets were euthanized and necropsied for sample collection at the end of each block. The PP diet contained 6.6% total non-starch polysaccharides (NSP), of which 2.5% were soluble NSP, whereas the WB diet contained 6.0% total NSP with 0.9% soluble NSP. Diet viscosity was higher for PP than WB (2.61 vs. 1.02 mPa·s). Statistical analysis was performed using a linear mixed model with diet as a fixed effect and pen and litter as random effects. No differences were observed in apparent ileal digestibility (AID) of dry matter between diets. AID of crude protein tended to be higher ($P = 0.078$) for WB (58.5%) compared with PP (53.3%). In contrast, AID of NSP was markedly higher for PP than WB (16.7 vs. 0.1%; $P < 0.001$). Ileal digesta viscosity was also higher for PP than WB (3.27 vs. 1.50 mPa·s; $P < 0.001$). In conclusion, P. palmata showed increased ileal NSP digestibility, likely due to its higher soluble fibre content, but also increased digesta viscosity, which may have limited crude protein digestibility. These results indicate a trade-off between fibre degradation and nutrient utilisation when using soluble fibre-rich seaweed in diets for post-weaning piglets. The research was conducted in the PIG-PARADIGM project, funded by the Novo Nordisk Foundation under the grant number NNFSA210073688.

Early-Life Robustness: Hematological and Immune Pathways Linking Pre-Weaning Growth to Post-Weaning Diarrhea Treatment

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Abstract Piglets with weaker constitutions are prone to severe post-weaning diarrhea (PWD), often requiring antibiotic intervention. This study aimed to elucidate the intrinsic mechanisms underlying early-life robustness in piglets and to identify key factors influencing PWD and subsequent antibiotic use. A total of 2.073 Danish crossbred Landrace Yorkshire Duroc (LYD) commercial piglets were included in the study. After data quality control, the final sample size for individual analyses ranged from 1.931 to 2.073 animals per phenotype. Complete blood cell count was collected at 25 days of age (one day before weaning). A comprehensive analysis integrating causal mediation analysis, genome-wide association studies (GWAS) and CatBoost classification modeling was conducted. The results revealed a significant negative correlation between pre-weaning relative weight (RW) and the need for antibiotic treatment after weaning (correlation coefficient = –0.344). Building on this observation, we applied causal mediation analysis to dissect physiological mechanisms mediating the relationship between RW and PWD treatment. Sex, batch, pre-weaning diarrhea indicator, and genetic principal components PC1 – PC5 were used as covariates in the causal mediation analysis. Hematological parameters, including hematocrit (HCT), measured hemoglobin (meas_HGB), and calculated hemoglobin (calc_HGB), mediated 6–9% of the total effect ($P < 2.2 \times 10^{-16}$). Immune parameters, including neutrophil percentage (per_Neut), lymphocyte percentage (per_Lym), and the neutrophil-to-lymphocyte ratio (NLR), mediated 2.5–4% of the association ($P < 2.2 \times 10^{-16}$). These mediating effects were stronger in slow-growing piglets than in fast-growing piglets. In the GWAS analysis, sex and batch were fitted as fixed effects for HCT, measHGB, calcHGB, and RW, whereas sex, pen, and pre-weaning diarrhea treatment were fitted as fixed effects for PWD treatment and NLR. In all GWAS models, the genomic relationship matrix was included to account for genetic relatedness. Genome-wide significant quantitative trait loci (QTLs) were identified for RW and several significant mediators. Finally, we evaluated the predictive performance of single nucleotide polymorphisms (SNPs) markers associated with significant mediators. A prediction model built using the relevant SNPs achieved an area under the precision-recall curve (PR-AUC) of 0.204, representing a 1.57-fold improvement over the baseline prevalence (0.129). Despite this improvement, the overall predictive ability remained moderate, indicating that additional informative genetic markers are still needed to improve prediction accuracy. These findings suggest that genetic variation, reflected in early-life hematological robustness, contributes to variation in susceptibility to PWD and may serve as biomarkers for growth performance and disease resilience. The research was conducted in the PIG-PARADIGM project, funded by the Novo Nordisk Foundation under the grant number NNFSA210073688.

Deciphering the intestinal pig microbiome and its function in early life

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The gut microbiome plays a central role in piglet development, resilience, and intestinal health, and is tightly interconnected with the developing host as well as its nutrition. The PIG-PARADIGM project funded by the Novo Nordisk Foundation in 2021 aims to improve the intestinal robustness and resilience in developing piglets by advancing fundamental knowledge of the pig gut microbiome and its interaction with nutrition and the host. These insights are expected to translate to strategies to reduce the dependence on antibiotics, thereby contributing to the global effort to mitigate antimicrobial resistance. Based on a large cohort study performed in Denmark with >2000 piglets, integrated efforts of the PIG-PARADIGM consortium are fully aligned and integrate host-microbiome multi-omics data to obtain a better understanding of the host-microbiome holobiont in the developing pig. To this end, microbiome-targeted activities set out to provide in-depth characterization of microbiome composition and function throughout the early life development of piglets from birth until 2 months of age. This aimed to establish the biogeography, i.e. the spatial and temporal dynamics, of the intestinal microbiome of the developing animals. While bacterial communities have been studied extensively, our analyses explicitly include all members of the intestinal microbiome, including bacteria and archaea, fungi and protozoa as well as viruses/phages. We use high-resolution metagenomics together with amplicon sequencing of taxonomic marker sequences (16S and 18S rRNA genes and ITS sequences) to map microbial biogeography across gut regions and developmental stages, map bacteria-host, and bacteria-microeukaryote correlations paired with dedicated virome analyses to uncover bacteriophage–bacteria correlations, providing the basis for the generation of hypotheses regarding possible causative interactions between different players within the gut microbiome – host holobiont. Furthermore, we aimed to identify pre-weaning prognostic microbiome biomarkers that allow prediction of antibiotic-treatment-requiring postweaning diarrhea (PWD) disease in piglets. To this end, animals were stratified based on phenotypic characteristics into those that are i) robust (no PWD) (ii) resilient (self-resolving PWD) and (iii) antibiotic-treated (antibiotic-treatment-requiring PWD). Results will be presented that were obtained by multivariate statistics and machine learning techniques used on three types of datasets (a) microbiota composition using 16S rRNA gene profiling; (b) weight dynamics; (c) blood parameters, to generate combinatorial predictive models to identify prognostic biomarkers. Beyond its relevance for swine health, this work will advance fundamental understanding of microbial succession, cross-kingdom interactions, and microbiome functional maturation. The findings could inform microbiome-based interventions, such as targeted bacteriophage applications or early-life microbial modulation, contributing to more robust livestock, reduced antibiotic use, and improved food system sustainability.
The research was conducted in the PIG-PARADIGM project, funded by the Novo Nordisk Foundation under the grant number NNFSA210073688.

Species-level faecal microbiota development from suckling to 25 days postweaning in piglets: response to pre-weaning nutritional supplementation assessed by shotgun metagenomics

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Early life gut microbiota influences postweaning resilience in pigs. Nutritional supplementation during lactation is increasingly adopted, yet its impact on microbiome development remains understudied. We hypothesized that pre-weaning supplementation promotes a more resilient gut microbiota. Piglets from 12 hyperprolific sow litters (standardized to 15 piglets) were allocated by litter to Control (n=6 litters; no supplementation) or Supplemented (n=6 litters; milk replacer on d 3–11, liquid creep feed from d 12 to weaning at d 24). Fecal samples from two piglets per litter were collected longitudinally before (11, 18, and 24 d of age), and after weaning (3, 7, 14, and 25 d postweaning). Shotgun metagenomes (Illumina NovaSeq X Plus; 2×150 bp; ~20M reads) were profiled using Sylph against GTDB-R220-C200. Species-level relative abundance and prevalence were modelled with MaAsLin3 (pre-weaning supplementation [treatment], sampling day [time], and their interaction as fixed effects; sow/pig as random effects). Shannon alpha diversity increased with age ($p<0.001$), with a trend for higher diversity in supplemented piglets ($p=0.052$) and no treatment × time interaction. Time was the dominant driver of community structure (Bray–Curtis; $R^2=0.34$, $p=0.001$), with a minor but significant treatment effect ($R^2=0.008$, $p=0.001$) and no treatment × time interaction. For species abundance, 22 species showed treatment × time interactions ($q<0.1$), notably two methanogens (Methanobrevibacter A sp900769095, Methanomethylophilus alvus) and a few uncharacterized Bacteroidota (Prevotellaceae, Muribaculaceae) had higher abundance in supplemented piglets on day 11 of age. For prevalence, 14 species showed earlier pre-weaning colonization in supplemented piglets, notably for five Prevotella species, Anaerovibrio slackiae, and two Vescimonas species. One hundred forty-three species showed a significant main effect of time ($q < 0.1$) on relative abundance, and 133 species on prevalence. Hierarchical clustering of abundance resolved at least 3 clusters, which we describe as steady colonizers (44 species), neonatal decliners (43 species), and weaning bloomers (31 species). Clustering based on prevalence showed that species were either progressively acquired over time (65 species) or were initially widespread but declined by day 14 postweaning (40 species). Cross-referencing clusters showed that decreases in abundance generally preceded losses in prevalence, consistent with progressive displacement during microbiome succession. Supplementation increased (treatment main effect: $q<0.1$) the relative abundance of Prevotella sp002251365, four Cryptobacteroides species, Romboutsia timonensis, and Methanobrevibacter A sp022775905, while Lactobacillus amylovorus, Limosilactobacillus mucosae, and Vescimonas sp900313295 were reduced. For prevalence, L. mucosae was reduced, whereas two uncharacterized species (UBA4334 [Bacteroidaceae] and CAG-103 [Oscillospiraceae]) were more prevalent in supplemented piglets. Pre-weaning supplementation preserved successional dynamics while influencing specific taxa in abundance and prevalence, with an overall trend towards higher alpha diversity, suggesting earlier establishment of a mature-like gut microbiota. Strain-level and functional characterization will further clarify these shifts and their potential relation to postweaning gut health. This work is part of the PIG-PARADIGM project funded by the Novo Nordisk Foundation (NNFSA210073688).

Non Digestible Carbohydrate Structure Differentially Modulates Responses to ETEC F18 in Weaned Pigs

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Post-weaning diarrhoea (PWD) caused by enterotoxigenic Escherichia coli (ETEC) F18 remains a major challenge in pig production, particularly following restrictions on zinc oxide and antibiotic use. Modulation of the non-digestible carbohydrate (NDC) fraction is a promising dietary strategy to enhance gut resilience. This study evaluated arabinoxylan (AX), xylo-oligosaccharide (XOS), and chito-oligosaccharide (COS) ingredients in an ETEC F18 pig challenge model. We hypothesised that AX, XOS, and COS would enhance resistance to ETEC F18 infection and reduce disease severity compared with the Control-ETEC diet. Eighty piglets were allocated to five groups: a non-challenged Control group and four ETEC-challenged groups (Control-ETEC, AX-ETEC, XOS-ETEC, COS-ETEC). The Control diet was based on rice with added oat hull meal and sugar beet fibre as complex NDC sources. In the supplemented diets, 4 % of oat hull and sugar beet fibre were replaced by AX, XOS, or COS. Piglets were orally inoculated with ETEC F18 on days 3 and 4 post-weaning (PW). Growth performance, faecal consistency, faecal dry matter (DM), ETEC shedding, and plasma acute-phase proteins (haptoglobin and PIG-MAP) were monitored over 21 days PW. Data were analysed in R (v4.3.3) using ANOVA and mixed-effects models, with diet and sampling day as fixed effects and block, pen, and piglet as random effects. Differences were considered significant at $P < 0.05$. Of the challenged groups with added NDCs, COS-ETEC showed the closest profile to the non-challenged Control group, regarding faeces consistency and DM%. During the acute post-infection phase (days 2 – 6 post-challenge), the estimated mean probability of diarrhoea based on faecal score remained below ~ 50 % in COS-ETEC, whereas it reached ~ 50 – 100 % in the other challenged groups. In general, due to high variation, significant differences between the COS-ETEC and the two groups receiving AX and XOS were only seen in a few cases. Despite these results, the dietary addition of COS did not reduce ETEC F18 shedding ($P > 0.05$) as compared to the Control-ETEC group, indicating that its anti-diarrhoeal effect was not mediated by direct pathogen suppression. In contrast, the AX-ETEC group displayed a profile closest to the Control-ETEC regarding faecal score, DM%, and estimated probability of diarrhoea based on both parameters. Moreover, the faecal ETEC F18 shedding was numerically highest compared to the other groups during most of the study period. For the acute-phase proteins, the haptoglobin levels on days 2 and 4 post-challenge were lowest in the XOS-group ($P < 0.05$), intermediate in the AX-ETEC and COS-ETEC groups, and highest in the Control and Control-ETEC groups, and with PIG-MAP unaffected across treatments ($P > 0.05$). Daily body weight gain for the whole period was highest for the non-challenge Control group, lowest ($P < 0.05$) for the three ETEC challenge groups with added NDC ingredients, and intermediate in the Control-ETEC. The same trend was observed for daily feed intake at the pen level. These results indicate that NDC ingredients with different structural characteristics differentially modulate host responses to ETEC F18. Although COS-ETEC did not reduce E. coli shedding, it improved faecal consistency and dry matter, suggesting that its effects are driven by strengthened gut resilience rather than bactericidal action. Accordingly, NDC structure and composition represent a key determinant in developing nutritional strategies to control PWD. Keywords: prebiotic, ETEC F18, post-weaning diarrhoea, non-digestible carbohydrates, gut health The research conducted in PIG-PARADIGM project, funded by the Novo Nordisk Foundation, grant number NNFSA210073688

Effects of Dietary L-glutamate, L-aspartate, and Their Combination on Growth Performance, Diarrhea Severity, and Amino Acid Digestibility of Weaned Piglets Challenged with Enterotoxigenic Escherichia coli

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L-glutamate and L-aspartate are functional amino acids (AA) essential for intestinal epithelial functions and physiological metabolism. This study aimed to investigate the effects of L-glutamate, L-aspartate, and their combination on growth performance, severity of diarrhea, and AA digestibility of weaned piglets challenged with enterotoxigenic Escherichia coli (ETEC). Sixty-five weaned pigs (7.59 ± 1.11 kg BW) were randomly assigned to one of five treatments (n=13). The dietary treatments included a control (CON), CON with 1% glutamate (GLU), CON with 1% aspartate (ASP), CON with 1% glutamate and 1% aspartate combination (GAC), and CON with 50 mg/kg carbadox (ABX) supplementation. All pigs were orally challenged with F18 ETEC for three consecutive days at the dose of 10^{10} CFU/dose/day. The study period lasted for four weeks, with a 7-day adaptation period and 21 days post-inoculation (PI). Body weights, feed consumption, and daily diarrhea scores were recorded throughout the experiment. Fecal samples were collected on d -7 and d 0 before ETEC inoculation, and on d 2, d 5, d 7, d 10, d 14, d 18, and d 21 PI to confirm the presence and dynamics of fecal β-hemolytic coliforms. Ileal digesta samples were collected on d 21 PI for AA digestibility analysis. All data were analyzed using ANOVA, except that diarrhea frequency was analyzed by the Chi-square test, with the individual pig as the experimental unit. The research was conducted in the PIG-PARADIGM project, funded by the Novo Nordisk Foundation under the grant number NNFSA210073688. The results revealed notable benefits associated with GLU, ASP, or GAC supplementation. Compared with CON, pigs fed ASP or ABX had increased ($P < 0.05$) body weight on d 14 PI, and pigs fed ASP, GAC, or ABX had increased ($P < 0.05$) final body weight on d 21 PI. Pigs fed ASP or ABX also enhanced ($P < 0.05$) average daily gain from d -7 to d 14 PI, compared with CON. GLU, ASP, GAC, or ABX supplementation increased ($P < 0.05$) average daily gain from d 0 to 21 PI, compared with CON. Additionally, GLU, ASP, GAC, or ABX supplementation improved ($P < 0.05$) average daily gain from d -7 to d 21 PI, compared with CON. ASP or ABX supplementation also increased ($P < 0.05$) average daily feed intake from d 0 to d 14 PI, compared with CON. Furthermore, supplementing with GLU, ASP, GAC, or ABX enhanced ($P < 0.05$) gain:feed ratio from d -7 to d 21 PI and from d 0 to d 21 PI, compared with CON. Moreover, pigs fed GLU, ASP, or ABX had reduced ($P < 0.05$) diarrhea frequency compared with CON (15.24 to 20% vs. 29.65%). In addition, pigs fed ASP, GAC, or ABX had reduced ($P < 0.05$) fecal β-hemolytic shedding on d 7 PI compared with CON. Apparent ileal digestibility (AID) of dry matter differed among treatments on d 21 PI, with pigs fed ASP showing the greatest ($P < 0.05$) digestibility value compared with the other groups. Additionally, pigs fed GAC had greater ($P < 0.05$) AID of lysine, aspartate, and glutamate than CON. Pigs fed GAC also showed greater ($P < 0.05$) SID of lysine, aspartate, and glutamate compared with CON. Moreover, pigs fed ASP had higher ($P < 0.05$) SID of aspartate compared with CON and ABX. In conclusion, dietary supplementation of L-glutamate, L-aspartate, or their combination enhanced growth performance, reduced diarrhea, and improved amino acid digestibility in ETEC-challenged piglets, supporting improved disease resistance during the post-weaning period.

Dietary protein and amino acid levels affect the gut metabolome and intestinal epithelial function in weaned piglets

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High dietary crude protein (CP) can enhance growth performance in weaned piglets but concurrently increases the risk of post-weaning diarrhea (PWD) by altering microbiota composition and increasing the metabolites from protein fermentation. A pig production trial recently demonstrated that supplementing a high-CP diet (22%) with surplus essential amino acids (EAA), including 35% extra crystalline lysine-HCl, with corresponding increases in methionine (32%), threonine (62%), tryptophan (22%), and valine (67%), reduced diarrhea treatment days by 50%, achieving an efficacy comparable to medicinal zinc oxide. Building on these findings, the aim of this study was to advance mechanistic understanding of how high-CP diets supplemented with surplus EAA influence gut health in weaned piglets. We hypothesized that excessive protein fermentation compromises gut health, while surplus EAA can modulate gut metabolome and mitigate these negative effects. This study included an in vivo animal experiment and in vitro cell assays. A total of 120 piglets were assigned to three dietary treatments: low-protein (15% CP; LP), high-protein (25% CP; HP), and high-protein with extra EAA (25% CP + surplus EAA; HP+EAA). The experiment was conducted in five blocks, each with 24 piglets allocated to six pens (four per pen; two pens per treatment). One piglet per pen was slaughtered on days 7 and 28 post-weaning (PW) for digesta collection from the distal small intestine and colon. Freeze-dried digesta was analyzed by untargeted metabolomics. Cell-based assays using porcine jejunal (IPEC-J2) and colonic (PoCo83-3) cell lines, as well as intestinal ileal and colonic organoids evaluated the effect of digesta and selected protein fermentation-derived compounds on cell viability and barrier function. Linear mixed-effects models were applied with treatment as a fixed effect and block as a random effect. Piglets fed the HP and HP+EAA diets, with significantly lower level of metabolites related to purine, fatty acid, and bile acid metabolism ($p<0.05$), exhibited distinct digesta metabolome profiles in the colon compared with piglets fed the LP diet. The purine nucleotide adenosine monophosphate (AMP) was present at higher levels in the HP+EAA group than in the HP group, indicating that surplus EAA supplementation modulates purine metabolism with enhanced purine salvage pathways that may increase nucleotides availability, thereby supporting colonocyte energy metabolism and anti-inflammatory responses. Lactobacillus genus was more abundant in the HP+EAA group, suggesting enhanced utilization of purine nucleosides and derivatives by these bacteria. In vitro cell line assays demonstrated that digesta from piglets fed the LP diet caused significantly less damage, measured as viability, to intestinal epithelial cells than digesta from piglets fed the HP and HP+EAA diets. Among several tested metabolites, p-cresol, ammonia, and hydrogen sulfide showed the most potent detrimental effects at physiologically relevant concentrations, whereas inosine and AMP stimulated epithelial proliferation. Organoid experiments showed that digesta from the HP group reduced viability more than that from the LP group, while surplus EAA partially alleviated this reduction. In conclusion, although metabolomics did not fully explain the reduction in PWD observed in vivo, the data suggested that purine metabolism-derived metabolites could partially contribute to this beneficial effect. Further, the organoid model more closely reflected in vivo responses than the cell lines. Funded by the Novo Nordisk Foundation (PIG-PARADIGM, NNFSA210073688).

Genetic Determinants of Gut Microbial Genera Abundance and Presence in Growing Pigs

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Gut bacterial communities play a crucial role in host physiology and are strongly associated with disease resilience, immune competence, and feed efficiency in pigs. Variation in microbial composition has been linked to economically important production traits as well as resilience to infectious and environmental challenges. Understanding the host genetic architecture underlying microbial community structure may therefore provide novel targets for improving animal health and performance. In this study, we investigated the genetic determinants of gut bacterial genera abundance and presence in a crossbred (Landrace x Yorkshire) x Duroc pig population (n=564). Microbial profiles were generated using 16S rRNA amplicon sequencing, and genotypes were derived using the Neogen 50k SNP panel. We performed genome-wide association analyses using a local-ancestry-aware framework conceptually similar to Tractor (https://github.com/Atkinson-Lab/Tractor), allowing estimation of ancestry-specific genetic effects and enhancing QTL detection in admixed populations. We identified 38 genome-wide significant QTLs associated with abundance/presence of 11 bacterial genera. Genes located near the lead SNPs were significantly enriched for the Gene Ontology terms "cell–cell junction" and "plasma membrane region," suggesting that the identified QTLs may influence bacterial composition through the modulation host–microbe adhesion. Notably, we observed distinct ancestry-specific effects at several loci, highlighting the importance of modeling local genomic background in crossbred populations. These findings demonstrate that host genetic variation contributes substantially to gut microbial composition in pigs. Due to standardized diets and controlled environmental conditions in livestock systems, pig populations offer a powerful model for detecting microbiome-associated QTLs with reduced environmental noise. Insights gained from such systems may facilitate the identification of conserved host–microbiome genetic interactions and inform similar studies in humans, where dietary and environmental heterogeneity often complicate discovery. Overall, our results provide new insight into the host's genetic architecture of gut microbial communities and establish a foundation for microbiome-targeted breeding strategies aimed at improving disease robustness and feed efficiency.

Stakeholder and consumer perspectives on animal welfare improvements in meat production

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Understanding stakeholder and consumer perceptions across the farm-to-fork value chain is essential for implementing effective animal welfare strategies. This study employed advanced analytical methods to identify barriers and motivators influencing adoption of welfare-friendly practices. This research investigated value chain actors' motivators and constraints using Topic Modelling and assessed EU consumer purchase intentions and willingness to pay using Structural Equation Modelling. Fifty stakeholder interviews conducted between September 2023 and January 2024 were analysed using Latent Dirichlet Allocation (LDA) Topic Modelling integrated with Large Language Model (LLM; Falcon-180B). The LDA algorithm identified latent themes through text preprocessing, treating documents as topic mixtures. Optimal topic numbers were determined through coherence score maximization. The LLM enabled objective categorization, identifying 26 key motivators and barriers across three domains. A consumer survey (n=5,064) tested two theoretical frameworks using Partial Least Squares Structural Equation Modelling (PLS-SEM). The Theory of Planned Behaviour (TPB) and Value-Belief-Norm (VBN) theory were evaluated for reliability, validity, and model fit. Comparative predictive performance was assessed using Cross-Validated Predictive Ability Test (CV-PAT) and Bayesian Information Criterion (BIC). Modelling of the interviews' data identified enhanced public awareness, financial incentives, regulatory frameworks, and certification schemes as primary motivators, while financial constraints, insufficient awareness, inconsistent regulations, and operational difficulties emerged as significant barriers. Survey analysis demonstrated positive attitudes with mean purchase intention of 3.58 (SD=1.06) and mean willingness to pay of 2.89 (SD=1.18). Trust in certification schemes ranged from 3.64 to 3.70. The TPB model explained 40.3% of purchase intention variance and 18.0% of willingness to pay variance, with attitude (β=0.366, $p<0.001$), subjective norm (β=0.276, $p<0.001$), and perceived behavioural control (β=0.197, $p<0.001$) as significant predictors. The VBN model explained 39.1% of intention variance and 23.7% of willingness to pay variance, with personal norms showing strongest influence (β=0.625 for purchase intention; β=0.487 for willingness to pay, both $p<0.001$), preceded by ascription of responsibility (β=0.561, $p<0.001$) and general awareness of consequences (β=0.502, $p<0.001$). Both models demonstrated excellent fit (TPB: GoF=0.461, SRMR=0.078; VBN: GoF=0.467, SRMR=0.055). Cross-validation confirmed TPB superiority for purchase intention prediction (average loss 0.790 versus 1.145, t=23.977, $p<0.001$), while VBN demonstrated superiority for willingness to pay prediction (BIC: -1353.456 versus -971.562). The integration of Topic Modelling with Large Language Model capabilities identified barriers and drivers influencing welfare-friendly practice adoption. Structural equation modelling revealed distinct psychological pathways, with attitude-based mechanisms dominating purchase intention formation while moral-value systems primarily drive willingness to pay premium prices. These findings indicate that effective market development requires targeted interventions addressing identified barriers and leveraging moral-ethical motivations in communication strategies.

Animal welfare and biosecurity on farm: are there conflicts?

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While biosecurity and animal welfare are widely understood to be essential for sustainable livestock production, tensions can arise when measures to reduce risks for pathogen transmission limit welfare opportunities, or when welfare-enhancing practices increase the likelihood of pathogen dissemination. As part of the Horizon Europe BIOSECURE project, we have explored farm decision-makers, welfare and biosecurity experts' perspectives on the relationship between biosecurity and animal welfare, with a particular focus on situations where the two may come into conflict and eliciting potential solutions for those conflicts. The study was divided in two steps. First, semi-structured interviews with the main farm-level decision-makers were done between May and December 2025. Nine poultry farmers in Ireland, 9 dairy farmers in Sweden and 10 pig veterinarians in Spain were interviewed. Participants were selected to reflect a range of herd sizes and management systems across each sector. Vignettes, developed based on previous input from livestock experts, were used to prompt the discussion. One vignette was common between all sectors (access to outdoor rearing) while the others were sector specific (e.g. barn systems for layers, slow growing breeds for broilers, enrichment material for pigs, and group pens for dairy calves). Secondly, online workshops (done in collaboration with the Horizon Europe aWISH project and the Swedish Center for Animal Welfare) targeting European biosecurity and welfare experts were organised for each species to present the results of the interviews and elicit their perspective in terms of potential conflicts and what solutions should be implemented. Attendance per workshop ranged from 23 to 38 with a near even split between biosecurity and welfare experts. During the workshop they were split into smaller groups and each group dealt with a potential conflict as per interviews' results. Interviews and workshops were recorded and analysed using thematic analysis to identify key themes. From the analysis there were common themes between sectors and farm decision-makers and experts: animal welfare and biosecurity were frequently described as dependent on and essential to each other. Outdoor access was considered "essential" for welfare but poses challenges for biosecurity. Risk mitigation options, such as fencing, were suggested to minimise exposure to wildlife. While solutions were found for many of the conflicts identified, it was acknowledged that some are not easy to implement on farm as they are resource intensive (e.g. extra labour, access to materials and more land, and new/adaptation of buildings) and vary with the different context, productions systems and sectors within countries and across Europe. Farm-specific supports, including knowledge transfer and flexible policies, were identified as key as a strategy of "one-size-fits-all" is not feasible. This study has identified both intuitive as well as non-intuitive conflicts between biosecurity and animal welfare by decision-makers at farm level, and biosecurity and welfare experts and local context appeared differently relevant for each sector and country. For some conflicts ready to use solutions could be identified whereas others may require policy changes or price compensation to accommodate extra costs.

Two frameworks for expert-based livestock welfare scoring: A comparative analysis

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Livestock welfare assessments typically incorporate a large number of welfare measures. Although multiple individual measures may be relevant to specific stakeholders (e.g. farmer or slaughterhouse), many applications, such as certification schemes, require a single aggregate welfare index. Consequently, the aggregation method not only affects how welfare is calculated for each stakeholder, but also determines the relative importance assigned to the different measures. This study compares two welfare elicitation and aggregation strategies, evaluating the resulting welfare indices and identifying the strengths and weaknesses of each approach. The first strategy, the indicator-based approach, corresponds to weighting each indicator explicitly, whereas the situation-based approach derives implicit weights from expert preferences between pairs of welfare situations (i.e., sets of indicators presented with different severity levels) using a discrete choice experiment framework. As part of the aWISH project, both approaches were evaluated via two case studies, focusing on broiler and pig welfare assessment at the slaughterhouse. For each species and approach, expert input was collected through online surveys. The broiler surveys included 11 welfare indicators (26/27 expert responses across rounds), whereas the pig surveys included 14 indicators (21/28 responses). The indicator-based survey elicited the perceived welfare severity and relevance of each indicator, whereas the situation-based survey included 30 comparisons, requiring experts to indicate the better welfare and quantify the difference in welfare. While data analysis is ongoing, preliminary results indicate poor to moderate inter-assessor agreement for the indicator-based approach (ICC: 0.40-0.52), with a greater disagreement for the low-severity levels of welfare indicators compared to the high-severity levels of indicators. The situation-based approach shows slight to moderate agreement when experts express a preference between two situations (Fleiss' κ: 0.19-0.46), and poor to good agreement when quantifying the magnitude of differences between welfare situations (ICC: 0.31-0.79). Notably, agreement between experts is not consistently higher or lower across species when comparing both approaches. Despite restricting participation to welfare experts, preliminary findings suggest limited consensus among experts under both approaches. Further comparative analysis will determine how these methodological differences translate into aggregate welfare scores and whether the limited agreement is due to true disagreement or the methodology of surveying. This research was performed within the framework of the Horizon Europe project aWISH (Grant agreement ID: 101060818).

Economic impact of enhanced pig welfare systems on Austrian finishing farms using information from automated monitoring

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Improving farm animal welfare has become a central objective of European agricultural policy, yet its economic implication at farm level remains insufficiently quantified. The EU project aWish focuses on automated monitoring of animal-based indicators at slaughterhouses to improve the welfare of broiler chickens and fattening pigs during each phase of production. During an aWish stakeholder workshop in Germany, participants discussed the feasibility and economic viability of keeping pigs with intact tails. Financial incentives were a recurring theme. Within aWish, the economic impact of farm-level management changes (enrichment, stocking density, barn design), using tail length, carcass classification and veterinary data from PigInspector (sensor installed at the slaughterhouse), has been explored at an Austrian pilot company. In Austria, voluntary welfare management systems such as 'Mehr Tierwohl – Gut' – (TW60) and 'Mehr Tierwohl – Sehr Gut' (TW100) require substantially more space per pig and additional measures (e.g., bedded resting area, and in TW100 GMO-free feed, intact tails, outdoor access and castration under anesthesia and analgesia), going beyond EU Directive 2008/120/EC and national minimum standards. However, evidence on how these management systems affect production costs and profitability across different farm sizes is still limited. The objective of this study is to quantify the economic impact of farm-level management changes on Austrian pilot farms implementing TW100, using information by automated monitoring data, compared with Austrian legal minimum requirements and EU Directive 2008/120/EC (Stevenson, 2012) in small, medium and large commercial finishing-pig farms. Baseline data were collected in 2024 from six pig farms in Upper Austria using semi-structured questionnaires developed within the TIPI-CAL (Technology Impact and Policy Impact Calculations) framework and validated with project partners and experts. Based on these data, three representative average farms were constructed to protect commercial confidentiality. Information on TW100 was obtained through an online interview with a farmer already implementing this system. Percentage changes in key indicators were applied to the representative farms. A TW60 scenario was modelled to capture intermediate animal-welfare requirements. The TIPI-CAL model (Chibanda et al., 2020) was used to assess the impact of the alternative welfare systems on production costs and farm profitability. Preliminary results indicate that TW60 and TW100 increase fixed and variable production costs per pig place, mainly due to lower stocking densities, higher labour inputs and additional investments in housing and enrichment. These welfare systems can improve technical performance, provided that returns compensate for the additional expenses. The net income effect varies by farm size: larger farms benefit more from economies of scale, while smaller farms face proportionally higher cost increases. In conclusion, feedbacks from automated monitoring at the slaughterhouse enables continuous improvements of animal welfare practices at the farm level. Enhanced welfare management systems such as TW60 and TW100 can substantially improve animal-welfare conditions but tend to raise production costs, especially in smaller farms, unless compensated by price premiums or targeted policy support. These findings are relevant for policy makers, supply-chain actors and farmers when designing incentives and labelling schemes for higher-welfare pig production.

How to evaluate animal welfare improving initiatives on regional and national level?

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Animal welfare improving initiatives (AWII) can be implemented by different private or public operators and at different levels (regional, national, supranational). To draw conclusions about the effects of AWII on actual animal welfare, sufficient monitoring data need to be available and accessible for analysis. Ideally, such data would be obtained from automated, cost-efficient, and reliable monitoring systems at the slaughterhouse and would correctly reflect the animals' welfare at different production stages. One of the aims of the aWISH project is to identify AWII for pigs and broilers on regional and national level and to evaluate their effects on animal welfare based on the data collected in the aWISH pilot slaughterhouses before and after implementation of the AWII, by comparing different groups of operators, or in comparison with historical records from national data repositories or other data sources. First, we developed four scenarios for these evaluations: a) the AWII has been implemented before the start of the aWISH project, data have been collected before its implementation and are available for an evaluative comparison from private-, national- or research repositories; b) the AWII has been implemented before the start of the aWISH project, suitable data before its implementation are not available, but data collected from different groups of operators during the aWISH project can be used for an evaluative comparison; c) the AWII is implemented during the aWISH project and data have been collected in the aWISH project before and after the implementation; and d) the AWII is implemented after the completion of the aWISH project, data have been collected during the project, are stored in the aWISH data platform, and can serve as 'before data' for future evaluations of the AWII. Second, we conducted an inventory to select suitable AWII for an evaluation within scenario a)-c). This consisted of a literature search and of an interactive multi-actor session. We identified three major challenges based on this inventory: the large volume and variety of AWII, data availability, quality, and accessibility. In our presentation, we will use examples of promising AWII to illustrate scenario a)-c). We found that in many cases, data that would allow for an evaluation of AWII are not yet (routinely) collected and recorded. In cases where such data are available, challenges arise with regard to data quality and access. For initially promising datasets, it turned out that at present the quality of data in terms of accuracy, reliability, and level of detail is insufficient to draw potential conclusions about the effect of an AWII on actual animal welfare. In addition, researchers need to overcome serious barriers (e.g., approval of legal data sharing agreements) to get access to data from external parties. These findings highlight the importance of the aWISH project for scenario d). The automatic collection of high-quality data in the pilot slaughterhouses and their recording in the aWISH data platform are a promising starting point. The structures established within the aWISH project will enable a more feasible evaluation of future AWII. Therefore, these structures need to remain operational after completion of the project and need to be expanded to include additional operators, production chains, and countries.

From animal-based welfare indicators to EU policy integration: lessons from the aWISH project

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The increasing recognition of animals as sentient beings has strengthened the scientific and policy interest in animal-based welfare indicators (AWIs). While substantial scientific knowledge exists on the development and validation of AWIs for pigs and broilers, less attention has been paid to their operationalisation within EU regulatory frameworks. This gap between scientific development and policy integration presents a critical challenge for the ongoing revision of EU animal welfare legislation. This contribution presents policy-oriented findings from the Horizon Europe project aWISH (Animal Welfare Indicators at the Slaughterhouse), focusing on the applicability and integration of AWIs into EU-level governance. The aim is to analyse how validated animal-based indicators can be embedded within legislative, inspection, and assurance systems in a manner that is scientifically robust, harmonised, and practically feasible across Member States. Drawing on project outputs and a high-level multi-stakeholder policy workshops involving representatives from the European Commission (DG SANTE and DG AGRI), Member State authorities, academia, civil society, and the livestock sector, we identify three core implementation challenges: (1) defining implementation-ready indicators that are measurable, cost-efficient, and scalable; (2) ensuring harmonised measurement, calibration, and reporting across Member States; and (3) embedding AWIs within coherent policy channels rather than parallel voluntary systems. Findings indicate that slaughterhouse-based data collection offers particular potential for centralisation, comparability, and digitalisation of welfare monitoring. However, effective integration requires clear definitions, common measurement protocols, baseline thresholds or tools to support their establishment, and alignment with the existing Common Agricultural Policy, inspection, and private assurance data systems. AWIs must complement minimum legal standards and be supported by guidance, training, and financial mechanisms to avoid disproportionate burdens on farmers and competent authorities. We propose a staged policy pathway for EU uptake of AWIs: short-term piloting and refinement; medium-term harmonisation and protocol development, potentially supported by EU Reference Centres; and long-term embedding within legislative and enforcement frameworks. This approach aims to strengthen outcome-based welfare governance while maintaining feasibility within diverse European livestock systems. These findings contribute to ongoing debates on outcome-oriented regulation in animal production and provide concrete recommendations for integrating scientifically validated AWIs into EU animal welfare reform.

A systematic review of sensor technologies for automated welfare assessment of broilers

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Animal welfare is a multidimensional concept covering many different aspects of the life of an animal, as a consequence, a variety and large number of indicators and measures are needed to assess welfare. Because of the time and effort needed to assess animal welfare, automatic registrations of indicators have a great potential. Within the European project aWISH, a systematic review of available animal based measures of broiler welfare was done. The indicators are divided into the five domains (Good feeding, Good housing, Good health, Appropriate behaviour and the Mental domain). In total, 65 measures for broilers were found. The majority of indicators (65%) were found for on-farm animal welfare challenges. While most welfare indicators were related to challenges occurring on-farm, most of them were measured at slaughter. Good health was the most represented domain with approximately 80% of indicators. The validity of the indicators that were measured was in most cases supported by literature. In the literature, there is less information about the repeatability and generality of the measures (e.g. possible differences between breeds or sexes). In the second part of the project a search was done for existing technological methodologies for measuring animal welfare indicators. Once the technologies were identified, experts were surveyed to complete the information about the technology identified to assess AWIs and their validation status, with different stakeholders related to whole meat production, including the livestock sector, academia, government, NGOs, and industry and retail. Most of the available technologies focus on animal welfare-related indicators (e.g. weight), rather than indicators specifically developed for animal welfare purposes. There are, however, a number of noteworthy exceptions, e.g. sensors for alarm call detection, assessment of gait score, foot pad dermatitis and hock burns.

Validation of an automated system for at-slaughter assessment of footpad dermatitis and hock burn in broiler chickens

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The automated assessment of footpad dermatitis (FPD) and hock burn via computer vision at the slaughterline could allow continuous monitoring of these important welfare indicators in broiler chickens. Such a system can be used to monitor the occurrence of FPD and hock burn over subsequent flocks and allow bench marking at different aggregation levels (farms, chains, countries, etcetera). This study aimed to compare the performance of a camera system (ChickenCheck software, CLK GmbH) against three human assessors. FPD (4 point scale based on relative size of the lesions) and hock burn (3 point scale based on absolute size of the lesions) were scored on both the left and right foot/hock according to severity from images captured by the camera system. A total of 50 images (100 feet/hocks) were used in the training dataset used to train the human assessors and 500 images (1,000 feet/hocks) were used in the validation dataset to determine measures of agreement and reliability for the discrete scoring system. Additionally, 100 images were used for lesion annotation (200 feet/hock) to determine the agreement between the camera system and the human assessors in capturing the total area of the foot pad or hock affected by lesions. Despite showing substantial to good intra-observer reliability (kappa: 0.78-0.98) and moderate to substantial inter-observer reliability (kappa: 0.46-0.66) during training, the human assessors showed fair to moderate agreement (kappa: 0.33-0.47) for FPD and hock burn when the dataset included a larger number of images with greater variation in lesion severity and appearance. However, the human assessors showed moderate to substantial agreement with the scores assigned by the camera system (0.60-0.70). More importantly, human assessors and camera system indicated a similar size of lesions on the footpad (R^2: 0.93) and hock burn (R^2: 0.97), suggesting that lesion size is correctly annotated by the camera system. However, the small but systematic differences found in the size of the affected area of FPD (+2.47%) and hock burn (-0.38 cm^2) by the camera system compared to the human assessors need to be addressed to further improve the performance of the automated system. A validated system for automatic assessment of FPD and hock burn at slaughter allows continuous monitoring of all birds that are slaughtered and is a valuable tool to improve broiler welfare. This study is part of the aWISH project. The aWISH ('Animal Welfare Indicators at the SlaughterHouse') project has received funding from the European Union's Horizon Europe Research and Innovation Program under grant agreement no. 101060818.

A molecular and biochemical reference approach for validation of vision systems that automatically record catching damage in broiler chickens at slaughter

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Catching damage, defined as lesions occurring during catching and associated preslaughter handling of broiler chickens, represents a significant challenge for animal welfare and carcass quality. Automated vision systems are increasingly implemented in slaughterhouses to detect carcass lesions; however, their validation is still largely based on macroscopic appearance or inspectors' subjective visual evaluations. Visual assessment, either human or camera-based, alone does not allow reliable determination of when a lesion occurred, making it difficult to distinguish between lesions arising during rearing or during catching, crating, transport or processing. Determining the age of lesions is essential both for accurate animal welfare evaluation and for production chain participants. The aim of this study was to develop a molecular and biochemical reference methodology that allows further training of the vision system to correctly recognize the age of lesions detected at slaughter. The approach was based on the established principle that mechanical injury induces a temporally ordered local response (inflammation), including activation of pro-inflammatory pathways, subsequent recruitment of immune cells, and later extracellular matrix remodelling and tissue repair. In parallel, injured tissue undergo progressive oxidative and enzymatic degradation, generating colour changes that reflect the stage of tissue response. Together, these processes are expected to produce measurable, time-dependent molecular and biochemical signatures in injured tissue. Sampling was conducted in a commercial slaughterhouse equipped with an automatic vision-based detection system (ChickenCheck software, CLK GmbH). Tissue was collected from carcasses classified by the system as presenting catching damage lesions. Gene expression profiling is applied to quantify transcripts of early inflammatory cytokines (e.g., L1β, Il6, Tnf-α), intermediate chemokines (e.g., CXCL8, CCL2), and late remodeling/fibrosis markers (e.g., MmP9, Tgfb1) using RT-qPCR. In parallel, biochemical analyses are conducted to determine hemoglobin-related parameters by UV-Vis spectrophotometry and concentrations of heme degradation products (biliverdin, bilirubin and conjugates) using HPLC or LC-MS/MS. Integrated statistical modelling, including mixed-effects and multivariate analyses is used to derive biologically based lesion-age categories. Analyses are expected to identify distinct molecular and biochemical patterns corresponding to different stages of catching damage lesions. Final laboratory analyses are currently underway and full results are expected prior to the conference presentation. This interdisciplinary approach links poultry welfare science, molecular pathology, and processing technology. It supports standardization of catching damage lesions assessment and contributes to the development of objective tools for welfare monitoring and quality control in the poultry industry.

Toward operational heat stress monitoring in poultry transport: a CFD-trained surrogate model

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Broiler chickens transported by road in summer are highly vulnerable to heat stress, with important welfare and economic implications. A previous Computational Fluid Dynamics (CFD) study have successfully characterized thermal heterogeneity inside transport vehicles, but simulation times of several hours make these approaches impractical for operational decision-making. This study developed a fast surrogate model that predicts both the spatial distribution of Effective Temperature (ET) inside transport vehicles and the time-dependent physiological response, enabling real-time estimation of stress severity across the load. A parametric CFD model was built using OpenFOAM® to simulate airflow, heat, and moisture transfer within a transport trailer. Broilers were represented as a porous medium with Ergun-derived resistance coefficients, while metabolic heat production followed CIGR (Commission International du Génie Rural, climatization of animal houses, 1984) recommendations partitioned into sensible and latent components. A Sobol sequence with rejection sampling generated 100 physically constrained scenarios spanning ambient temperature (20–40°C), relative humidity (limited by realistic western european specific humidity bounds of 5–22 g/kg), stocking density (150–230 cm²/kg), and average bird weight (1.2–3.0 kg). From each generated CFD solution, 20 ET quantiles (from q01 to q99.95) were extracted, capturing not only average conditions but also the critical upper tail where the most vulnerable animals are exposed. Kriging surrogate models were trained independently for each quantile using normalized inputs. Leave-One-Out Cross-Validation demonstrated strong predictive performance ($R^2 > 0.96$). This allows real-time reconstruction of the ET distribution across the load, though spatial localization within the vehicle is lost. By coupling this reconstructed distribution with a physiological response law linking ET to core body temperature rise, the surrogate enables rapid prediction of stress category proportions (Normal, Alert, Danger, Emergency) as a function of exposure time. The surrogate model enables rapid evaluation of mitigation strategies, supporting operational decisions such as adjusting loading density or scheduling transport during cooler periods. Future field validation will quantify the relationship between predicted stress categories and mortality outcomes. Ultimately, this approach provides a practical framework for balancing animal welfare, logistics, and economic constraints in real-time.

Impact of bird placement on Airflow Resistance in laying hen Transport Crates using a Porous Media Approach

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Animal welfare during transport to the slaughterhouse may be challenged by inappropriate thermal conditions, potentially inducing thermal stress. There is limited knowledge of how temperature is distributed in laying hens transport vehicles. This project aims to investigate temperature distributions inside transport vehicles by applying Computational Fluid Dynamics (CFD) simulations. This study, as part of the project, is to investigate the porous medium modelling of the large number of laying hens and crates present in the transport vehicles to significantly reduce mesh numbers so that CFD simulations are feasible with our available computational resources. The objective of this study was to identify the resistance coefficients of porous medium modelling for crates with animals considering three animal placements (three scenarios designed based on observations) within crates. The actual geometry of laying hens and crates' walls were created and first simulated in CFD modelling so the correlation between the pressure drops and air speeds (five levels changing from 0.25 m/s to 1.25 m/s) could be developed along three directions of Cartesian coordinate system. This allowed us to derive resistance coefficients of porous medium regions. Then the same computational domain was used while the actual geometrical domain of laying hens and crates' walls were replaced by porous medium regions and simulations under the same air speeds were conducted. The pressure drop obtained from these simulations were compared with pressure drops from the previous simulations at the first step. The results showed that the porous medium modelling can well generate the pressure drop with discrepancies ranging from 0% to 5% for air speeds larger or equal to 0.5 m/s and up to 11% for air speed of 0.25 m/s. The different placements of laying hens could influence the resistance coefficients; thus, the pressure drops to a certain degree. The results of this study can be applied by researchers and engineers focusing on CFD modelling of laying hens transport vehicles in order to further predict the temperature field within the vehicles under different climatic conditions.

Optimizing Pig Welfare Through Digital Transparency: A Multimodal Sensor System for Real-Time Transport Monitoring

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Live animal transport represents a critical welfare vulnerability in pig production, characterized by opacity and significant variability in stress and injury outcomes. While digital traceability now extends to breeding and on-farm phases, transport remains a poorly monitored black box, undermining value chain integrity and consumer trust in ethical practices. This study addresses this gap by developing a compartment specific sensor system for real-time welfare assessment during transit. The architecture integrates commercial smartphones including built in accelerometers, gyroscopes, cameras, microphones, GPS and low cost Bluetooth tags to capture spatially resolved data across transport bays. The key innovations include compartment level granularity, hybrid hardware, and multimodal fusion. Granularity is achieved by acquiring data per bay, which detects intra vehicle welfare disparities due to density, positioning, and microclimate variations. Hybrid hardware is realised by using smartphones as both sensor nodes and data hubs, leveraging their ubiquitous processing capabilities, while Bluetooth tags supplement environmental sensing of temperature, humidity, and pressure. Multimodal fusion is obtained by simultaneous capture of environmental, motion, behavioral and geospatial data to enable real time welfare indexing. Four interdependent streams are monitored. The environmental stream tracks temperature, humidity and pressure, to quantify thermal risk. The journey-dynamics stream contains of acceleration and vibration, correlating motion with physical stress. The animal-indicator stream employs bay-specific cameras and microphones to capture vocalisations, posture and visible injuries, which are analysed with machine-learning algorithms. The geospatial context stream integrates GPS with data on road quality, traffic and weather, situating each welfare event within its broader context. Data from the streams are temporally aligned, features such as vibration spectra and acoustic entropy are extracted, and supervised machine learning generates real-time welfare indices (e.g., stress level, injury risk). The system creates concrete impacts for four stakeholder groups. For producers and transport operators, it reduces mortality, enables optimized loading patterns and improves operational efficiency through proactive interventions. For slaughterhouses, it provides verifiable welfare data for quality assurance and regulatory compliance. For consumers and brands, transparent, evidence based information about ethical handling enhances trust and enables market differentiation. For regulators, the platform supplies objective high resolution data that supports evidence based welfare enforcement. Compared with existing solutions, the system replaces single-point monitoring with bay-specific analysis, revealing intra vehicle welfare differences that were previously hidden. It maximizes cost efficiency by reusing smartphones instead of installing dedicated sensors. Finally, it shifts welfare management from retrospective audits to real time monitoring. The proposed system transforms pig transport into a digitally transparent, welfare optimized segment. By delivering compartment level, real time metrics, it enables evidence-based interventions that enhance animal health, operational efficiency, and regulatory compliance. This framework establishes a scalable model for ethical meat production, demonstrating how digital innovation bridges welfare science and industrial practice. Future work will focus on field validation and standardized welfare indicators for industry adoption.

"Best Practices" to improve broiler welfare – scientific experts' vs. poultry farmers' perspectives

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As part of the EU Horizon project "aWISH" (Animal Welfare Indicators at the Slaughterhouse), so called "Best Practice Guides" to improve animal welfare in poultry farming were developed. To ensure both scientific accuracy as well as suitability of the proposed "Best Practices", animal welfare experts and poultry farmers were consulted in two surveys and asked to evaluate the measures. First, a literature review was carried out to identify Best Practices to reduce the occurrence of footpad dermatitis/hock burns (FDP/HB) and catching damage (CD) in broilers. Subsequently, 15 identified Best Practices for FDP/HB and 10 for CD were integrated into two surveys: one was sent out to scientific experts (aWISH partners, the project's expert panel and external experts from 21 countries), the other to German poultry farmers. Participants were asked to rate the proposed Best Practices on a scale from 0% (unsuitable) to 100% (very suitable). Data analysis, including a mixed linear regression model, was performed with the software R. Overall, 37 completed questionnaires were collected from experts and 60 from farmers. For FPD/HB, the measures "Good ventilation in stables (e.g. ventilation rate)" (experts: 91.0% vs. farmers: 94.0%, $p>0.05$) and "Height of waterline adjusted to animal size" (80.0% vs. 86.5%, $p>0.05$) received the highest scores in both groups. Significant differences between farmers and experts were detected for "Slow growing breeds" (76.0% vs. 25.0%, $p<0.001$) and "Litter management" (77.5% vs. 46%, $p<0.001$). Experts rated all measures to reduce catching damage at ≥70%, while farmers were far more critical towards practices relating to upright catching, such as "Carrying broilers upright" (73.5% vs. 20.0%; $p<0,001$) or "Not picking broilers up by their wings or legs" (85.0% vs. 25.0%, $p<0.001$). Experts selected "Careful handling" as the top Best Practice (99.0% vs. 83.0%, $p>0.05$), while farmers favoured "Low light intensity/blue or red light during catching" (80.0% vs. 100.0%, $p>0.05$). Experts and farmers agreed on the effectiveness of most of the proposed measures. Significant differences emerged regarding practices that pose a challenge for practical implementation on farms. It is therefore important to consider practical constraints like costs, feasibility and workload when developing guides to improve animal welfare to ensure acceptance of the measures among farmers. This project has received funding from the European Union's Horizon Europe Research and Innovation Program under Grant Agreement No. 101060818.

Effect of season on pasture composition, intake, and health in horses

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In addition to the plant cell wall polysaccharides, the aerial parts of grassland plants contain non-structural carbohydrates (NSC): starches and soluble carbohydrates including simple sugars and fructans. The NSC content in plants can account for more than half of the dry matter, which can trigger metabolic diseases in grazing horses. Epidemiologically, seasonality is observed in the incidence of metabolic laminitis, which could reflect seasonal variation in the chemical composition of pasture vegetation and ingested biomass. Some of these variations are known at the botanical species level, but in multispecies grassland ecosystem these are poorly understood. Similarly, the selection and preference for plant species by horses in such grazing environments across different seasons and the resulting impact on their metabolism are unknown. This project aims to fill these gaps. Twenty-four healthy horses (14±1 yo, 701±83 kg) grazing on multispecies pastures (n=138 species documented at the start of the project) were monitored in spring (SP, May), summer (SU, August) and autumn (AU, October). During the 4 weeks preceeding each sampling, horses were grazing exclusively on pasture and received no additional feed. In SP, SU and AU, 4 samples of pasture biomass were collected for chemical analysis. At the same time, feces and blood samples were collected from the horses. Plant DNA present in the feces was extracted and sequenced (ITS2 rRNA sequencing) to determine the composition of the ingested diet. Serum fructosamine (SF) was used as a medium-term indicator of average glycaemia over the previous weeks. The effect of the season on chemical composition of the pastures, SF, and the richness and diversity of plants ingested was measured by ANOVA, as was the effect on the structure of the plant communities ingested by PERMANOVA (SAS). Relation between SF and the chemical composition of the pasture was assessed by ACP (R). Profound changes were observed in the chemical composition of pasture throughout the seasons (Table 1). The structure of the plant communities ingested also varied greatly (P<0.001), with a particularly distinctive structure in SP. This was due mostly to plants from the family Brassicaceae, which accounted for 42.9% of the plant DNA in feces in SP, compared to 1.8% and 2.3% in SU and AU (P<0.001). Conversely, the abundance of Fabaceae increased from 3.5% in SP to 27.0% in SU and 20.7% in AU (P<0.001). The richness and diversity of plants ingested was higher in SU than in the other seasons (on average 67 species in SP, 77 in SU, 63 in AU, P<0.001; Shannon index: 1.96 in SP; 2.35 in SU, 2.05 in AU, P<0.001). Concentration of SF was higher (P=0.038) in AU compared to SP and SU (in µmol/L: SP=324.8, SU=328.2, AU=355.3), but poorly related to chemical components of the pasture. When kept in multispecies pastures, horses ingest a varied diet that changes with the seasons. While NSC concentration was the lowest in AU, SF concentration peaked during this season, suggesting that other parameters may influence this parameter in horses. Understanding these factors of variation should promote practices that reduce the risk of metabolic disturbances, thereby contributing to the health of grazing horses.

Table 1. Plant biomass composition (in %OM) per season (means and SE)

	Spring	**Summer**	**Autumn**	**Season effect** ***P-value***
Crude protein	13.0±1.2[c]	14.8±1.4[b]	21.9±2.9[a]	<0.001
Cellulose	31.1±1.1[b]	33.1±1.7[a]	23.3±2.9[c]	<0.001
Hemicellulose	20.4±3.0[c]	22.4±2.6[b]	26.3±2.0[a]	<0.001
Lignin	7.1±0.6[b]	9.8±1.0[a]	7.2±0.8[b]	<0.001
Starch	2.6±0.6[a]	1.3±0.4[b]	1.1±0.3[b]	<0.001
Simple sugars	8.3±1.0[a]	6.3±1.0[b]	5.6±0.8[c]	<0.001
Total NSC	25.2±3.2[a]	16.4±3.0[b]	17.5±4.6[b]	<0.001

The use of shade by grazing horses

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Periods of high summer temperatures are increasingly considered a management issue for grazing horses. In this study, we investigated short-term behavioural adjustments of horses when provided with shaded areas under summer pasture conditions. This experiment was conducted at the experimental farm of Chamberet (France) in 2023 and 2024. In total, 54 adult non-pregnant and non-lactating mares aged 5-8 years (2023: N=25; 2024 N=29) were included. Behavioural observations were performed in July and August. Mares were separated in three balanced groups grazing on two plots subdivided into three adjacent plots (1.20-1.50ha) corresponding to three treatments: shade (S), with permanent access to a grove; partial shade (PS), provided by trees along the plot edges; and without shade (WS). Environmental conditions were characterized using data from on-site meteorological station. Thermal load was estimated using the THW index (temperature humidity wind, in °C). Observation sessions were classified into four THW categories: <20°C (N=20), 20-25°C (N=28), 25-30°C (N=26) and >30°C (N=22). Behavioural states (grazing, rest while standing, lateral rest, recumbency rest, walk, observation, vigilance, positive/negative social interactions and insect repletion) were recorded using instantaneous scan-sampling (1 record every 10 mn) three days a week with four daily time slots (08:30-10:00, 10:30-12:00, 13:30-15:00, 15:30-17:00). At each scan, the position of the mare (in shade or in sun) was also noted. Animal performance was lastly monitored by weighing the mares at the beginning and end of each observation week. Effect of THW and observation time on behaviours expression was studied using Kruskal-Wallis test with the Dunn test as post-hoc when needed. Across all observations, grazing and rest while standing dominated the time-budget. Significant differences between treatments emerged only when THW index exceeded 25°C. S mares spend more time grazing than mares in PS and WS treatments both at 25-30 °C (S: 62.3±5.4%; PS: 49.3±17.7%; WS: 50.3±10.4%; p-value<0.05) and >30°C (S: 60.3±11.2%; PS: 40.0±12.7%; WS: 44.4±8.6%; p-value<0.05). Correspondingly, resting while standing increased in PS and WS mares. When THW index is between 25-30°C, WS mares (36.1±11.7%) rested more (p-value<0.05) than S (24.3±8.1%) and PS (23.6±7.4%) mares. When THW index exceeded 30°C, the difference between S and WS mares increased (21.3±6.8% vs 39.1±8.9%, respectively; p-value<0.05), and a significant difference also appeared between S and PS mares (21.3±6.8% vs 45.7±12.2%; p-value<0.05). However, further analyses are needed to determine whether these behavioural differences are directly associated with actual shade use. For PS mares, the amount of time spend in the shade depends on shade availability throughout the day, but when shade was present (15:30-17:00) its use was similar (36.8±17.1%) of that of S mares (40.4±13.2%; p-value=0.82). No significant difference in body weight was observed between treatments (from 552.9±26.5 kg to 569.1±23.6kg in 2023; from 528.2±26.9kg to 554.5±37.9kg in 2024). These results highlight short terms behavioural adjustments of grazing mares when facing increased thermal load. Access to shade appears to modulate these adjustments. However, interpretation remains cautious because treatments differed not only in shade availability but in shade structure (i.e. grove microclimate) and daily shade dynamics. In addition, only daytime observations were performed. The absence of difference in body weight suggests that behavioural compensation strategies may occur outside the observed periods.

Investigation of a pivotal age in the ageing of digestive function in horses.

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Elderly horses may experience health issues related to digestion and absorption, such as loss of body condition or colic. In this herbivore species, digestion in the hindgut and absorption of volatile fatty acids (VFA) through intestinal wall are crucial for energy provision and maintaining health. Digestion itself depends on the retention time (RT) of substrates and relies on microbial activity. With ageing, the alterations in the equine microbial ecosystem have only been described through linear relations and age groups comparisons. With the concept of a "pivotal age" (PA) – the age at which these changes accelerate – it is sought to anticipate the health issues associated with ageing. This study aimed to assess age-related changes in markers of digestive function, including RT, which has never been studied in this context. It was also examined whether the changes are linear or if PA can be demonstrated. A cross-sectional study was conducted with 48 horses without ingestive, digestive or metabolic diseases, evenly distributed between 4 and 33 years old. The horses were fed only hay ad libitum. To assess microbial activity, indirect proxies (VFA concentration and pH) were measured in faeces. Plasma acetate (C2) concentration and faecal dry matter (DM) were evaluated as markers of hindgut absorption. Total tract RT was measured as a reflect of the hindgut RT, by administering an indigestible marker (polypropylene balls) by nasogastric intubation on 24 horses. For 50 h, total faeces were collected and sieved to count the number of balls excreted. The mean RT (MRT) was considered as the time after which half of balls were recovered (t1/2, in h). The model was used to calculate t1/2. As the horses included in this study were of different breeds and sizes, a weight correction was applied. Four models with different complexity were tested for each variable: broken-line model (BLM; $Y = \alpha + \beta 1 \times (age - PA)(age<PA) + \beta 2 \times (age - PA)(age>PA)$), linear-plateau model (LPM; $Y = \alpha + \beta \times (age - PA)(age>PA)$), linear model (LM; $Y = \alpha + \beta \times age$) and intercept-only model (IOM; $Y = \alpha$). Models were compared based on their AICc values, the lower the better. Models with $\Delta AICc < 2$ were considered as equivalent. For MRT and plasma C2 concentration, the IOM had the lower AICc with a fixed intercept at 2.8±0.1 h.kg BW-1/3 and 0.93±0.04 nmol/µL, respectively. For fecal VFA concentration, pH and DM, the LPM had the best fit (Table 1) with a PA of 22±2 yrs, 19±3 yrs and 18±2 yrs and slopes of 2.72 mmol/L/yr, -0.07/yr, and 0.64 %/yr after the breakpoint, respectively. Using the chosen marker method, MRT did not vary with age. Thus, ageing of digestive function might be more related to changes in the composition and activity of the hindgut microbiota. Although this cross-sectional study cannot assess intra-individual ageing trajectories, its modelling approach could further explore microbial changes. A PA of around 20 yrs has been identified for the indirect proxies of microbial activity, consistent with the PA observed for one of the absorption markers (DM). Confirmation of age-related changes in absorption using more specific markers would be valuable. This modelling approach could also help identify trajectories of physiological functions during ageing and early contributors to frailty.

Table 1. AICc of models tested for different parameters

	Total fecal VFA	Fecal pH	Fecal DM	Plasma C2	MRT
BLM	224.1	-94.0	-337.7	FTC*	FTC*
LPM	221.9	-96.2	-340.1	FTC*	FTC*
LM	227.0	-92.1	-331.2	-110.5	-35.4
IOM	236.71	-78.7	-314.1	-111.3	-35.5

*FTC: Fail To Converge

Chicory as an Antiparasitic Driver: Shaping the Parasite–Microbiota–Host Triad in the Equine Holobiont

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Anthelmintic resistance in cyathostomins is a growing global threat to equine health and welfare, underscoring the need for sustainable, ecology-based parasite-control strategies. Chicory (Cichorium intybus, Puna II) has been proposed as an antiparasitic forage, yet its effects on the parasite–microbiota–host system remain uncharacterized. We conducted a longitudinal study to evaluate how chicory grazing influences parasitological outcomes, gut microbial communities, and host responses and behavior in horses. Twenty-six naturally infected 2-year-old Anglo-Arab foals were monitored over a 32-day grazing trial, with 13 horses grazing ~ 80% chicory-based sward and 13 grazing a permanent pasture. Both groups were followed concomitantly. Animals were monitored weekly for clinical and behavioral indicators, fecal egg counts, larval development, blood biomarkers (inflammation, immunity, metabolism), and high-resolution dynamics of the microbiota and nemabiome. Statistical analyses combined linear and generalized mixed-effects models for parasitological, physiological, immunological, and behavioral variables, and multivariate ecological approaches for microbiota and nemabiome data (PERMANOVA, β-dispersion, differential abundance tests, and longitudinal stability metrics). Physiological parameters remained stable across treatments, with no differences in body weight or major clinical markers. Immune profiles showed limited variation, although IL-4 increased in chicory-fed horses, suggesting subtle Th2 modulation. Behavioral assessments confirmed the absence of discomfort. Feeding duration was similar between groups (≈18 h/day), and chicory-fed horses showed slightly greater displacement and more positive social interactions. Importantly, horses grazing chicory exhibited a marked reduction in cyathostomin egg excretion, accompanied by functional shifts in the intestinal ecosystem. While nemabiome composition remained stable in controls, the relative proportion of several cyathostomin species – notably Cyathostomum pateratum and multiple Cylicocyclus taxa (C. nassatus, C. ashworthi, C. leptostomus, C. insigne) – decreased in chicory-fed horses. A few taxa, including Cylicostephanus calicatus, C. minutus, and C. longibursatus, persisted or increased, indicating species-specific sensitivity to chicory rather than uniform suppression. Concomitantly, chicory induced profound ecological shifts in the gut microbiota, including reduced α-diversity, increased β-dispersion, and destabilized individual microbial trajectories. Several bacterial lineages, namely Oscillospiraceae, Clostridiaceae, Lachnospiraceae, and Bacteroidales, were differentially enriched in chicory-fed horses, indicating a functional reorganization of the intestinal environment. Together, these results show that while chicory reduces nematode fertility and reshapes nemabiome composition, it also induces substantial shifts in the gut microbiota and host responses whose overall impact is not necessarily beneficial. Chicory may offer ecological leverage for parasite control, though its broader effects on the holobiont warrant caution.

The maternal vertical transmission of the bacterial microbiota shapes the foal microbiota on the long-term

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Maternal intestinal microbiota is transmitted to the foal prenatally and postnatally via amniotic fluid, milk, and coprophagy. The microbiota of foals undergoes progressive maturation during the first months of life, leading to a structure similar to that observed in mares from 2 months of age. By this age, the foal microbiota has reached a stable state, yet it continues to evolve alongside the dietary transition until weaning. This study explores whether vertical bacterial transmission is merely a route of colonization of the immature foal gastrointestinal tract or whether it shapes the individual bacterial structure. Although mare-foal microbiota similarity has been investigated at the cohort level, information at the level of individual pairs remains limited. The aims of this study were to determine the degree of similarity of dam-foal pairs and to assess the contribution of the maternal microbiota to a distinct microbial signature in foals over time. Twelve French Trotter dam-foal pairs from a single farm and sharing common pastures were enrolled in the study. Fecal samples were collected from dams in May and November, and from foals at 2 (61±3 days, May-July) and 7 months (227±14 days, November-December). Foals were weaned gradually between 5 and 6 months (171±21 days). Bacterial community structure was characterized using 16S rRNA gene sequencing. Inter-individual diversity (β-diversity) was assessed using Bray-Curtis dissimilarity index (0=similar; 1=dissimilar). For each sampling period, the observed mean dissimilarity index between dams and their foals was compared against a null distribution generated from one million simulations of the mean index calculated from 12 randomly paired unrelated mare-foal or foal-foal combinations. Age-related differences in dam-foal similarity were evaluated using a paired Wilcoxon signed-rank test. Intra-individual diversity (α-diversity) was assessed using Shannon index. Pearson correlations were used to test linear relationships between dams and foals α-diversity. At 2 months, foals' bacterial microbiota was more similar to that of their own dams than to that of unrelated mares (0.684±0.101 vs 0.755±0.017, $p<0.0001$) or other foals (0.684±0.101 vs 0.725±0.015, $p=0.0022$). Similarly, at 7 months, foals' microbiota remained more similar to that of their own dams than to unrelated mares (0.585±0.015 vs 0.672±0.019, $p<0.0001$) or other foals (0.585±0.015 vs 0.653±0.018, $p<0.0001$). Dam-foal similarity was stronger at 7 compared to 2 months ($p=0.0024$). No correlation was observed between dam and foal α-diversity at 2 months ($r=0.503$, $p=0.0954$) but a significant positive one at 7 months ($r=0.775$, $p=0.0031$). At 2 months, foal microbiota is more similar to that of its own dams than to that of unrelated mares or other foals. This pattern persists after weaning, despite shared environmental exposure, highlighting a stronger maternal influence compared to environmental factors. Additionally, the results indicate that α-diversity is vertically transmitted. It would be interesting to investigate whether the resistance and resilience properties, often associated with α-diversity, are also transmitted. Optimizing maternal microbiota and potentially selecting breeding mares based on their microbial profile, may therefore represent a promising strategy to promote the health and performance of foals.

Effect of alkaline reduced water on gastrointestinal inflammation in horses

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Gastritis affecting the squamous and glandular mucosae can originate from starch fermentation in the stomach of horses. Respectively, starch bypassing antececal digestion ferments rapidly in the hindgut, which can lead to dysbiosis. Horses may exhibit diarrhea and systemic inflammation due to hindgut barrier permeability. Based on observations made in other species, drinking alkaline reduced water (ARW) may help reduce gastrointestinal inflammation. It is currently unclear whether horses willingly drink ARW and what effect it has on their health. Thus, this study aimed to evaluate voluntary consumption of ARW and assess its impact on gastrointestinal health in horses fed a high-starch diet. A longitudinal study composed of two periods (P1 and P2) of 4 weeks each was conducted on 12 geldings (French Trotter, 10±1 yo, 556±40 kg). Throughout the study, horses were fed a high-starch diet (1.8 g/kg BW starch/meal, twice daily) at an ingestion level of 2.0% BW, consisting of hay (61% DMI), rolled barley (29%), and wheat bran (10%). Horses were housed individually in boxes and had free access to water in 80L buckets to measure consumption. During P1, all received standard tap water. In P2, horses were divided into 2 groups: the control remained on tap water while the other received ARW (mixture of 2/3 tap water and 1/3 Xeral [Watair GmbH]). At the end of each period, blood was taken to count cells and assess electrolytes, gases, plasma acetate and lipopolysaccharides (LPS) concentration. Likewise, feces were collected to measure DM and calprotectin. On the last day of each period, lesions in both mucosae were assessed during gastroscopy. Microbiopsies were collected to evaluate vascular congestion, edema, crypt atrophy in the glandular mucosae (H&E coloration) and NF-κB expression in both mucosae (immunohistochemistry). Data from P2 were analyzed using the GLIMMIX procedure with values from P1 as covariates. During P2, horses consumed same amounts of both waters, confirming that ARW was readily accepted. Horses receiving ARW showed lower RBC (P=0.008), hematocrit (P=0.022) and hemoglobin (P=0.005), suggesting hemodilution, which might be related to the increased concentration of blood sodium (P=0.077). They also had higher base excess (P=0.048), probably resulting from the alkalinity of ARW (pH=11.2), which could be beneficial in cases of metabolic acidosis. No significant effect of ARW was observed on gastric or hindgut inflammation markers (Table 1). However, in horses receiving ARW, numerically lower scores for glandular vascular congestion and crypt atrophy, along with reduced NF-κB-positive surfaces in squamous mucosa, hypothesize decreased inflammation. The higher fecal DM and plasma acetate concentration suggest that ARW may also improve hindgut barrier integrity and absorption capacity. As such, the gastrointestinal benefits of ARW were not clearly demonstrated in horses experiencing subclinical, chronic dietary stress. However, this data supports further studies to assess the impact of ARW on animals facing acute stress or diseases.

Table 1: Effect of water drunk on markers of gastric and hindgut inflammation

	Control	ARW	SE	P-value
Glandular mucosa				
Histologic Scores1:				
– Congestion	1.69	1.23	0.20	0.140
– Edema	1.84	1.41	0.28	0.311
– Crypt atrophy	1.75	1.17	0.30	0.200
NF-κB (% of area)	3.71	2.95	1.10	0.639
Squamous mucosa				
NF-κB (% of area)	1.81	0.29	0.60	0.104
Feces				
DM (%)	23.4	24.6	0.6	0.203
Calprotectin (µg/g)	760	777	34	0.727
Plasma				
Acetate (nmol/µL)	0.54	0.68	0.07	0.180
Total LPS (pmol/mL)	476	423	38	0.363

1 0=Normal, 3=Severe

Horse feeding in relation to gastric health – nature does it best

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Feeding plays a crucial role in the etiology of gastrointestinal and metabolic diseases. In horses, many common diseases are related to feeding excessive amounts of soluble carbohydrates in cereal grain-rich meals, to which horses as grassland inhabitants are not well adapted. The objective was to assess changes in grades for equine squamous/glandular gastric disease (ESGD/EGGD) as well as biochemical and physicochemical parameters of gastric digesta in relation to the type of ration. In 24 horses (13 m/11 f), 11 ± 6.0 years, 471 ± 63.4 kg body weight (BW), gastric mucosa was examined by gastroscopy before a feeding period of at least 34 days and afterwards after dissection. The horses were on pasture 24 h/day (PST) or received hay ad libitum (HAY), hay plus oats at 1 or 2 g starch/kg BW/meal (OS1/OS2) in the stable (n = 4/8/6/6). Stabled horses had straw as bedding and daily paddock access. The horses were not exercised. The shift in grades from the initial to final examination was calculated. The grades were transformed into a binary trait for deterioration and odds ratios calculated with reference to PST. Dry matter (DM), swelling capacity (SC), water binding capacity (WBC), buffering capacity (BC), percentage of particles less than or equal 1 mm (Ple1), pH, chloride and mucus were determined in digesta of Pars nonglandularis/glandularis (PNG/PG) and gastrin in blood serum. In the stabled horses, last hay was offered 2 h and oats 1 h prior to sedation, euthanasia and sampling. The shift in ESGD and EGGD grades and changes in digesta constituents were analyzed using mixed linear models. Moreover, linear regression was carried out to unveil the effect of an oat meal in addition to hay ad libitum considering the supplementary levels of oats at 0, 1 and 2 g starch/kg BW/meal (HAY/OS1/OS2) for DM, WBC, SC, BC, Ple1, pH, chloride, mucus and gastrin. The shift in ESGD and EGGD grades differed among the rations ($p < 0.05$) and was specific in individual stomach sections. While horses benefited in the PST and HAY groups, deterioration in OS1 was observed exclusively in the antrum and in OS2 irrespective of the section. In relation to PST, odds ratios for horses to deteriorate in terms of mucosal lesions were 1.0 (HAY), 2.9 (OS1) and 20.2 (OS2; $p < 0.01$). Addition of oats led to considerable changes in biochemical and physicochemical parameters. Especially WBC was lower with oat feeding ($p < 0.001$). Digesta pH was highest in OS1 and OS2 ($p < 0.05$) and mucus concentration was upregulated ($p < 0.001$). Linear regression revealed that the changes resulting from additional oat feeding were primarily due to shifts in DM, WBC and Ple1 in PNG, and DM, mucus concentration, WBC, SC and Ple1 in PG ($p < 0.01$). For the other analytes, an effect of oat supplementation was not evident. Feeding concentrates seemed to disturb the natural sponge effect of digesta, whereupon gastric acids remain on the mucosal surface. This could be one major reason for lesions that occur both in the squamous and the glandular mucosa. Even a proportion of oats referring to 1 g starch/kg BW/meal cannot be fed without hesitation and twice this in one meal is assured detrimental for gastric health. The study was approved by Saxon Federal Administration Authority (TVV 63/21) and funded by the German Research Foundation (project no. 434510792).

Wash it all away - Evaluation of the efficiency of different cleaning methods on drinking water quality in automatic bowl drinkers for horses under practical conditions

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Water is an essential nutrient for maintaining physical functions and the health of horses and must always be available in sufficient quantity and quality. But there is neither a clear definition what is meant by sufficient water quality, nor is there a specific guidance on measures to maintain sufficient water quality. Therefore, the aims of the experimental study were to collect data on microbiological water quality in bowl drinkers under practical horse husbandry conditions and to compare different cleaning methods for keeping automatic bowl drinkers clean. A total of 30 bowl drinkers with tube valves used by horses kept in individual boxes at a horse farm in North Rhine-Westphalia were examined for the study. Firstly, a sample of the stagnant water was taken from each bowl drinker to determine the initial bacteria content. The following 5 cleaning methods were carried out in respectively n=6 bowl drinker: (a) scooping out the residual water by hand (control group), (b) wiping the surface by hand, (c) wiping with a paper towel, (d) cleaning with a dish brush, (e) cleaning with a brush and subsequent application of a commercially available chlorine-based disinfectant for use in horse husbandry. After cleaning, the bowl drinkers were emptied by hand and then refilled with fresh water to the previous level. Finally, a second sample per bowl drinker was taken from the fresh water immediately after refilling. All samples were microbiologically tested for the total aerobic count at 20°C and 37°C, as well as the content of coliform bacteria and Escherichia coli. The results were evaluated descriptively and are shown as mean ± SE or respective as Δ of the mean ± SE. Initial bacterial concentration varied between 4.0 and 7.48 log (10) cfu/mL for total aerobic count at 20°C and between 3.0 and 6.48 log (10) cfu/mL for total aerobic count at 37°C. Comparing the reduction for total aerobic count at 20°C of the different cleaning methods showed, that cleaning with the brush and subsequent disinfection (e) had the greatest effect (-1.57 ± 0.17 log (10) cfu/mL, followed by cleaning with a brush (d) (-0.85 ± 0.18 log (10) cfu/mL), scooping out the water by hand (a) (-0.75 ± 0.09 log (10) cfu/mL), wiping with a paper towel (c) (-0.54 ± 0.25 log (10) cfu/mL), and wiping the surface by hand (b) (-0.06 ± 0.26 log (10) cfu/mL). Total aerobic count at 37°C showed similar results. Coliform bacteria were detected in 13 bowl drinkers before cleaning. After cleaning, coliform bacteria only remained in 2 bowl drinkers, both which had been cleaned by (b) wiping the surface by hand. In all water samples E. coli was below the detection limit of 1.0 log (10) cfu/mL. Therefore, it can be concluded, that cleaning the drinkers using a cleaning tool is more effective than simply scooping out the water by hand. After comparing the effort and benefits, the use of a brush as a cleaning tool can be recommended for further studies. In order to keep the measures practice-oriented, regular cleaning with a clean rinsing brush appears to be advisable. In the case of illness, disinfectant should be used additionally. Furthermore, the study provides initial results on microbiological water quality parameters under practical conditions as a basis for defining sufficient water quality for horses. This research is part of the project Aqua Equidae, funded by the Gesellschaft zur Förderung der Wissenschaft um das Pferd eV (GWP).

Alternative control against Stomoxys calcitrans: efficacy assessment of a litter-desiccant on its life cycle

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Stomoxys calcitrans is a cosmopolitan hematophagous fly and a major pest of cattle and horses, also biting other mammals and birds. It impairs animal welfare, transmits pathogens and causes economic losses. Immature stages develop mainly in litter soiled with urine and feces, manure and feed refusals. Intensive chemical control of adult flies has led to insecticide resistance and, since then, current recommendations promote integrated pest management (IPM) combining repellents on animals, selective traps, parasitoid releases and improved litter hygiene. In cattle farming, farmers use litter drying and sanitizing agents to control the development of pathogenic microorganisms and improve long-term litter quality (e.g., humidity and pH). However, they also reported a decrease in the number of adult flies, although this has not been clearly measured yet. The aim of our study was to assess the efficacy of the litter desiccant Actisan 360® (Timac Agro, Saint-Malo, France), on the life cycle (egg laying and development of immature stages) of S. calcitrans under laboratory conditions. First, the egg-laying (or oviposition) preference of adult flies was tested between a treated and an untreated substrate. To this end, 100 adult flies (sex ratio 1:1) were placed in a cage containing two oviposition trays, one control and one treated with Actisan 360®. Flies were fed daily with cattle blood, and eggs laid on each tray were collected and counted. Measures were followed up until all flies had died. Six replicates were performed. Egg counts were compared between treatments using a negative binomial mixed model (with a significance threshold of $p < 0.05$). Secondly, the effect on immature stages development was assessed. Thus, six treated trays and two control trays each received 500 eggs (3000 and 1000 eggs total, respectively). Newly emerged adults were collected daily, and then the sex, weight and size were determined. The trial ended after two consecutive days without emergence. The remaining pupae were then collected from each tray, counted, weighed and measured. All data were analysed using generalised linear models, mixed linear models, ANCOVA and survival analysis ($p < 0.05$). Egg laying was significantly reduced on the treated substrate (mean reduction: 94%; $p < 0.001$), indicating a strong repulsive effect from the treatment substrate. Pupal formation decreased by 63.7% in the treated larval medium ($p < 0.001$), indicating reduced larval survival. However, no pupicidal effect ($p = 0.869$) or inhibition of adult emergence ($p = 0.375$) was observed. The size for adults and pupae did not vary between conditions. However, adults were significantly lighter in the treated group ($p < 0.01$), whereas pupae were significantly heavier ($p = 0.010$), possibly reflecting reduced larval competition within the treated substrate. In conclusion, this study demonstrates that Actisan 360® exerts a strong repellent effect on oviposition site selection, together with a significant larvicidal activity. These findings suggest that Actisan 360® could be integrated as a complementary tool within an environmentally friendly IPM strategy.

Case Study: Mineral Content of Trimmings from Different Hoof Parts Using TXRF in an Oldenburg Sport Horse With and Without Mineral Feed Supplement

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Nutritional supplements are common in horse feed to improve health and performance, including hoof stability. Several minerals such as zinc, copper and manganese are known to positively influence hoof health and are often supplemented in special formulations. Minerals act as co-factors or inhibitors in healthy hoof keratinization and mineral tracing could reveal if and how feed supplements impact hoof composition. Methods to verify mineral uptake and long-term incorporation from supplements into hoof material remain limited. The aim of this case study is twofold: it (1) evaluates potential variations in mineral uptake in different parts of the hoof such as wall, frog and sole over time with and without mineral supplementation and (2) investigates the feasibility of using Total Reflection X-ray Fluorescence (TXRF) to analyse mineral content in hoof trimmings. If feasible, a simpler handheld XRF device could be used in a next step to verify mineral composition of hoof material without costly laboratory analysis. The study is based on the case of an Oldenburg sport horse transferred from a standard diet optimized for sport horses with additional mineral supplementation including biotin, zinc, copper and manganese to a purely grass-fed diet with roughage for 6 months. Horse shoes from the front legs were removed 3 months after the transition. From that point onward, regular trimmings from the front left hoof were taken by a farrier using nippers for 18 months, specifically from the hoof tip, side wall, sole and frog. After the 6 month period, the horse was again transitioned to stable housing with feed supplements. Samples were taken at an average of 36 ± 6 days. All hoof samples were washed with ultrapure water (>21 MΩ/cm). The samples were milled in a cup mill using tungsten carbide cups and balls. Milled samples were stored in PP tubes under vacuum. 250 ± 1 mg per sample were acid digested in a microwave digestion system using HNO3 and measured in TXRF using molybdenum and tungsten excitation. The TXRF was calibrated using six reference materials and verified using ICP-OES. In the sole, concentrations for zinc, copper and manganese were 93.47 ± 14.01 ppm, 3.59 ± 1.86 ppm and 38.24 ± 36.26 ppm respectively. In the frog, concentrations were 35.81 ± 4.85 ppm (Zn), 2.98 ± 2.06 ppm (Cu) and 46.68 ± 38.32 ppm (Mn) and in the hoof wall 104.09 ± 33.30 ppm (Zn), 5.99 ± 3.61 ppm (Cu) and 90.44 ± 54.51 ppm (Mn). A one-way ANOVA showed no significant difference for zinc, copper and manganese in samples from the two feeding regimes in the sole. However, in the hoof wall differences were found for copper ($F = 10.449$, $p = 0.0045$) and manganese ($F = 12.7410$, $p = 0.0024$). In the frog differences were found only for zinc ($F = 11.4427$, $p = 0.0062$). The TXRF results were validated using reference material with acceptable recovery and variance, comparable to methods such as ICP-OES (R2: > 0.95) and showing similar ranges as reported in literature (Spörndly-Nees, 2023; Stanek, 2025; Litzke, 2020), demonstrating the feasibility of TXRF for mineral determination in horse hoof. The evaluation shows a significant reduction in copper and manganese concentrations in the hoof wall and zinc in the frog after switching from supplemented feed to a grass-fed diet, but not in the sole. This highlights the need to distinguish sample material in further analysis. This study is limited to a single-horse case with significant diet changes. Results should be verified with more individuals and extended to additional material such as mane and tail hair. The present results can serve as a basis to calibrate a simplified handheld XRF device to analyze more samples in situ.

Data science and innovation – from academic research to industrial applications

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In the Data Science team at CRV, we develop, test and productize data-informed solutions that can help farmers, business partners as well as serve internal CRV processes. For example, we have recently developed risk metrics based on milk fatty acids that alert farmers to cows with poor rumen health and high negative energy balance. To this end, we integrated existing data on milk production with milk fatty acids and tested the results in practice in collaboration with feed advisors. CRV has a long history of collecting data, and has fully committed to professionalizing its applications in the past decade. In this talk I will give an insight into some of the experiences I had in the past 2 years since my move to industry after 10 years in academic research. I will share some of the differences in doing independent academic and company-embedded research and dive into some key lessons I have learned. Topics will be for example the professional process of bringing innovations to the market, legal and other boundaries of commercial development, important hard and soft skills I had and I lacked from working in academia. I will share about some of the projects I have been involved in, and how we test new ideas in the market. I will also touch upon potential opportunities for more and better collaboration between industry and academia.

Practical application of PLF technologies in pasture based production systems, from academia to startups

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The European livestock research landscape has produced a substantial body of work in Precision Livestock Farming (PLF), yet the translation of academic prototypes into scalable, market-ready technologies remains limited. While crop-oriented agritech and food-tech sectors have attracted significant venture capital and deep-tech development, animal agriculture has comparatively lagged in the emergence of research-driven startups. This gap is particularly evident in extensive, pasture-based, and small-scale systems, where technological innovation often remains confined to experimental or pilot-stage deployments. Regrowth originated from applied academic research on pasture-based poultry and integrated agroecological livestock systems in central Italy. Initial development focused on a low-cost weight-on-weighing (WoW) platform designed to provide growth and welfare data within mobile and pasture environments. The early phase followed a conventional parameter-driven PLF logic used as a standard in PLF development. However, direct implementation across diverse contexts including upland systems in the Alps and Apennines and smallholder poultry farms in Kenya revealed that technical validity alone does not guarantee adoption. Instead, infrastructural constraints, user experience, and economic thresholds shaped real-world viability. Through iterative field engagement and human-centric design methodologies, Regrowth reframed its development process. Rather than positioning sensors as the primary innovation output, the project adopted an infrastructure-first architecture centered on edge computing, modular sensor integration, and offline functionality. This shift enabled adaptation across heterogeneous environments, from high-altitude transhumant grazing systems to low-infrastructure rural farms, demonstrating that technological flexibility is critical for scalability. The transition from academic initiative to startup entity provided reciprocal value to scientific research. Direct market exposure enabled rapid validation cycles, stress-testing of theoretical assumptions, and identification of adoption barriers that are rarely visible within controlled research environments. Entrepreneurial activities required the transfer of research competencies such as experimental design, systems thinking, data modeling, and validation protocols into product development, stakeholder communication, and sustainable business structuring. This process generated feedback loops that enriched ongoing research activities, aligning scientific inquiry more closely with field-level realities. Consumer and farmer engagement further shaped innovation pathways. Participatory discussions, co-design sessions, and on-farm prototyping highlighted that end users prioritize reliability, transparency, and incremental investment capacity over high-resolution physiological analytics. By embedding human-centric design principles into the core architecture, Regrowth sought to reduce the structural gap between academic innovation and operational livestock systems. This case study argues that fostering deep-tech development in animal agriculture requires stronger integration between research institutions and entrepreneurial mechanisms. Embedding market validation within early-stage scientific development can enhance both impact and scientific robustness. The Regrowth pathway illustrates how academic livestock research can evolve into a context-responsive, scalable innovation framework capable of serving diverse production environments while contributing back to the scientific ecosystem.

AgrifoodTEF after three years: Lessons learned by researchers providing innovation services to agrifood tech companies

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AgrifoodTEF aims to accelerate the adoption of Artificial Intelligence (AI) and robotics in the agrifood sector by providing access to testing and experimentation facilities across Europe. The project creates a collaborative environment where research organizations support companies by offering services such as testing, validation, and performance assessment of emerging technologies. In doing so, it strengthens the link between academic expertise and industrial innovation, positioning research institutions as providers of applied technological services. Since its launch in 2023, AgrifoodTEF has progressively developed a European ecosystem dedicated to support innovation. After 3 years, the project provides an opportunity to reflect on what researchers have learned from this new service-oriented approach. To date, more than 400 services have been delivered to over 200 companies, with an average value of about €13,000 per service. Most of these companies are European Small and Medium-sized Enterprises (SMEs). Thanks to the TEF funding mechanism, many of them benefited from financial support covering up to 100% of the service cost, enabling them to access high-level scientific expertise and experimental infrastructures. These services highlight key strengths of research institutions: advanced experimental infrastructures, multidisciplinary scientific expertise, and the ability to evaluate technologies across a wide range of environments. For companies developing AI or robotic solutions, AgrifoodTEF offers a reliable framework to test and validate innovations. Activities can range from simulation and laboratory experimentation to trials in controlled facilities and experimental farms. This allows companies to progressively assess and refine their technologies, making research organizations particularly valuable partners in preparing innovations for market deployment. However, this experience has highlighted several challenges. Moving from a traditional research role to that of a service provider for industry requires significant adjustments. Companies expect clear added value, rapid engagement, and concrete results, which often challenges the timelines and procedures of research organizations. Another important shift for researchers is adopting a more client-oriented posture: instead of primarily proposing and promoting their own scientific ideas, they must first listen to the company's expectations and needs, and tailor the service accordingly. Our capacity to evaluate the real market for these services is also challenged as AgrifoodTEF framework introduces a structural bias. The targeted audience is relatively narrow, focusing mainly on SMEs developing AI and robotics solutions, and many do not directly pay for the services because the costs are covered through the project's funding mechanism. While this support greatly facilitates access to research infrastructures, it also makes it difficult to assess the actual demand and willingness to pay for such services outside the project context. To ensure the long-term sustainability of service activities within research institutions, it is essential to continue working on the specific value researchers bring to industrial partners. It is equally important to better understand the needs of different types of companies—start-ups, SMEs, and large enterprises—which may require distinct types of services, collaboration models, and financing mechanisms. AgrifoodTEF thus not only supports technological innovation but also provides valuable lessons on how research organizations can adapt to effectively engage with industry and support the deployment of new technologies in the agrifood sector.

Development of an interactive sensor system for recording animal activity for intelligent, group-specific optimization of the barn environment in pig farming

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Modern pig farming is currently the focus of considerable public and political interest. There is a demand for the production of safe, high-quality food in conjunction with climate-friendly and resource-efficient production systems, while at the same time maintaining high animal welfare standards. At the same time, digitalization offers growing potential for livestock farmers through the management and bundling of data streams. Additionally, it also offers solutions to the growing demands in the upstream and downstream stages of the value chain. At the same time, the opportunities for pigs to express their natural exploratory behavior, which can be significantly improved to meet the needs of pigs, which are considered intelligent animals. The SmartPigHome project aimed to develop an interactive sensor system for pig farming to intervene in situations with agonistic behavior to reduce stress in pigs. Therefore, cameras and acoustic sensors should record animal activities and use this information to start an innovative "activity tool" on demand. The tool – consisting of buzzers, light and acoustic stimuli, and reward feeding – was designed to encourage natural exploratory behavior and to reduce aggressive behavior (e.g., tail biting). The core components were: optical recording of activity and lying behavior using 2D cameras and acoustic analysis to distinguish between positive and negative pig sounds – both signals serving as a trigger for an automatic intervention by sound and light scenes and a final reward feeding if a correct buzzer was used by a pig. Following preliminary studies on light colour preferences in pigs and the development and testing of SmartPigHome components on a laboratory scale with animals, a prototype of the interactive activity tool was tested under field conditions in a piggery with fattening pigs. Results: Acoustic and optical triggers were successfully combined to detect stressful situations. Initial tests showed that pigs can learn to use buzzers and that haptic stimuli in combination with feed proved to be particularly attractive. Challenges lay in the robustness of the hardware in the corrosive stable climate, variable lighting conditions, and the validation of the algorithms. Overall, the prototype of a SmartPigHome shows that a sensor- and AI-supported adaptive barn environment can be used under field conditions. Future developmental steps must lead to a reduction in system complexity and further optimization of technical components. The project "SmartPigHome" (28N602901) was funded by the German Federal Office for Agriculture and Food.

Automated detection of boar taint to enable sustainable pig production

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Pork is one of the most widely consumed animal proteins globally, with over 120 million tonnes produced annually worldwide in a market valued at around $300 billion. As demand for affordable, high-quality animal protein continues to grow, the pork industry faces increasing pressure to improve efficiency, sustainability, and animal welfare. One of the key challenges is the continued use of surgical castration of piglets to prevent boar taint, an unpleasant odour in cooked pork that occurs in a small percentage of entire male pigs. While raising entire male pigs is considered the most sustainable and welfare-friendly production system, the transition is limited by the lack of rapid and objective methods to identify tainted carcasses during slaughter. PigO FoodTech is a startup company, developing an automated solution for real-time classification of meat quality attributes, including boar taint, directly within industrial workflows. The system enables objective decision making at processing speed. With increasing regulatory pressure and rising expectations for animal welfare, the meat industry requires scalable tools for objective quality assessment. By enabling rapid detection and classification during processing, PigO FoodTech aims to support the transition toward more sustainable pig production while protecting product quality, operational efficiency, and consumer confidence.

From Compliance to Sustainable Continuous Improvement: Embedding AI-Enhanced Post-Mortem Assessment into a Structured Program to Advance Herd Health and Welfare in Pigs

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High-throughput slaughter inspection of pigs is primarily designed to ensure regulatory compliance, yet the large volume of routinely collected information also represents an underused resource for improving herd health and animal welfare. This contribution presents a co-innovation project between the Goldschmaus Group and SLA Software Logistik GmbH aimed at embedding AI-enhanced post-mortem assessment into a structured program for advancing herd health and animal welfare. The initiative forms part of the slaughterhouse's "Program for Animal Welfare and Animal Health," within which slaughter findings represent one important component of a broader set of advisory and improvement activities involving farmers, veterinarians, and regulatory authorities. Within this program, industrial AI-based computer vision systems were implemented directly in the slaughter process. These systems combine fully automated classification with assistant-supported assessment and structured image documentation. Nine standardized animal welfare indicators are collected per pig, such as ear condition, tail length, external injuries, and organ findings. This enables consistent collection of defined indicators for each animal and reduces the variability that can arise from high-frequency assessments, cognitively demanding examinations, and individual interpretations. The project was developed in close cooperation with the Veterinary Office of Cloppenburg and in dialogue with practicing veterinarians. Continuous image-based documentation enhances transparency, supports official inspection procedures, and provides a reliable basis for professional evaluation. Data generated within the slaughter process are aggregated and interpreted within the program's structured feedback processes, enabling earlier identification of recurring herd-level challenges and supporting targeted advisory discussions with farmers and herd veterinarians. As a result, advisory farm visits and veterinary consultations are increasingly guided by objective herd-level evidence rather than isolated findings. The implemented systems include automated welfare indicator detection as well as assistant-supported organ assessment, strengthening the linkage between slaughter findings and systematic herd health analysis by practicing veterinarians while also supporting veterinary inspectors in documenting and reviewing post-mortem findings under high-throughput conditions. This case illustrates how slaughterhouses can evolve into structured animal health partners within the livestock value chain. By harmonizing procedures and integrating AI-enhanced post-mortem assessment within advisory and regulatory collaboration, slaughter data can support targeted herd health interventions, reduce production losses, and contribute to more sustainable livestock production systems. The presentation will illustrate this approach using practical examples, showing how slaughter findings are translated into herd-level health management and targeted advisory interventions.

Fostering Data Sharing to Construct High-Quality Datasets: Unlocking AI Potential for Livestock Behavior Studies

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Common CCTV cameras can be transformed into automated livestock monitoring systems using convolutional neural networks (CNNs). This has been demonstrated in numerous studies with applications for animal welfare, health, behavior, and, more generally, precision livestock farming. Most studies rely on common CNN architectures, such as YOLO, which are available as open-source tools. Although YOLO features a powerful architecture and has proven to be generic and robust, its accuracy is often too low—meaning animals are not detected properly—for many specific livestock applications. Consequently, these models often require re-training for each specific farm condition, which limits their widespread use in animal studies. To unlock the full potential of CNNs for livestock monitoring, it is necessary to rely on robust models specifically designed to operate in diverse farm environments.To achieve this goal, one approach is to construct a high-quality dataset combining images of animals across a wide variety of conditions. Indeed, the primary focus should be diversity: including as many breeds, camera types, angles of view, lighting conditions, backgrounds, and animal colors as possible. Combined with high-quality annotations, this could lead to a trained YOLO model that could be used across virtually all livestock applications with little to no fine-tuning. Interestingly, such datasets likely already exist within the numerous "proof-of-concept" studies developed by researchers over the last decade. What is currently lacking is a willingness to share data, the infrastructure to do so, and a shared definition of labels.As we enter the AI era, the key role of data has been underlined numerous times. The successful development of AI tools, particularly for livestock, depends on our ability to collaborate and construct datasets that can serve as benchmarks for training and comparing new methods. The LIB (Livestock Images Behavior) project was initiated to foster this dynamic by constructing a comprehensive dataset for livestock detection. It serves as an example of how collaborative work can help overcome common challenges in the field. We hope it will stimulate computer vision applications for livestock and serve as a foundation for generalizing practices such as data sharing, infrastructure development, and standardized label definitions.While the long-term success of the project remains to be seen, LIB has already successfully collected nearly 50,000 images associated with over 800,000 labels. Specifically, 20,011 images were collected for small ruminants (including sheep and goats), 20,924 for pigs, 6,433 for cattle, and 1,712 for chickens. These images were shared by multiple institutions, primarily across Europe. The project's impact could be further enhanced by involving more institutions during the initial design phase to improve visibility and encourage institutional incentive policies.All images were labeled manually by two full-time employees over one year, including the location (bounding box) of the animal's body and head, as well as their posture (standing, lying, or transitioning). Annotation quality was verified by re-labeling a random subset of images, yielding a precision of 99.7% and a recall of 98.6%. The dataset will be publicly released alongside pre-trained YOLO models for detection and segmentation. Furthermore, it will include an Ultralytics-based solution to facilitate the analysis of multiple videos for livestock behavior monitoring. Specifically, it can be used to measure inter-individual distances, zone occupancy, and heat maps, with results exportable to CSV files for direct use in behavioral analysis.

Digital animal welfare data in pig production: Collection practices, data-sharing patterns and determinants of farmers' willingness to provide data

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Background Animal welfare has become a central concern in pig production with increasing demands for transparency from consumers, policymakers, and retailers. In response, digital documentation systems and precision livestock farming (PLF) technologies support data-based monitoring and reporting. However, their implementation depends on how animal welfare data are governed, shared, and perceived by farmers, while empirical evidence on farmers' perceptions and data-sharing intentions within PLF data ecosystems remains limited. Objectives This study investigates (1) how digital animal welfare data are collected and used on pig farms in Germany, (2) how farmers' willingness to provide such data, including perceived advantages and disadvantages, varies across potential data recipients, and (3) which factors drive or hinder data-sharing intentions within PLF-based data ecosystems. Methods From June to September 2024, an online survey of 103 German pig farmers was conducted within the "Transparency in Pig Production" project. Data were analysed using descriptive statistics, bivariate tests, and regression analysis based on validated constructs derived from an extended UTAUT model. Results Although animal welfare data are increasingly collected, digital evaluation and automation remain rare. The most strongly perceived advantages of providing digital animal welfare data are benchmarking opportunities, fewer on-farm inspections, and improved transparency, whereas key disadvantages relate to surveillance, loss of autonomy, and concerns about data security and privacy. Farmers' willingness to share data varies by the intended recipient, with low acceptance of data sharing with governmental institutions and higher acceptance for veterinarians and farm advisors, alongside a clear preference for anonymous and controlled data-sharing arrangements. Regression analysis reveals that positive attitudes towards digital animal welfare data and low technology-related anxiety are the strongest predictors of behavioural intention (to provide data), complemented by trust in data governance, attitudes towards animal welfare programs, and farm size (measured by number of fattening pigs). Notably, classic UTAUT constructs such as performance expectancy, effort expectancy, facilitating conditions, and social influence, as well as age and current animal welfare program participation, do not significantly influence farmers' willingness to provide such data. Discussion & Conclusion The findings show that the willingness to provide digital animal welfare data within PLF systems depends less on performance- and effort-related technology expectations and more on farmers´ trust in data governance arrangements and their perceived control over data sharing, indicating the need to shift PLF development from a technology-driven to a governance-oriented perspective. This underlines the importance of transparent data governance frameworks, robust data protection mechanisms, and clear rules on data access and control to enable trustworthy, interoperable, and widely accepted PLF data ecosystems that support responsible innovation in livestock production. These aspects have key implications for PLF adoption in European livestock production.

Building environment diagnostics in cattle farming in the Upper Rhine region

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Climate change in the Upper Rhine region will manifest as a rise in average temperatures of between 1.5 and 3.0°C by 2050, as well as an increased frequency of heatwaves. These conditions directly affect the sustainability of cattle farming systems. High-yielding dairy cows are particularly sensitive to elevated temperatures. The Temperature Humidity Index (THI), which combines temperature (T) and relative humidity (RH), is commonly used as a warning tool. Another climate index such as the Heat Load Index (HLI) was developed. Although less widely used than the THI, it is calculated based on relative humidity, wind speed (WS), and the impact of solar radiation. Consequently, to improve animal welfare and support cattle farmers in their operations, the Chamber of Agriculture Alsace and the LKV Baden Württemberg, in collaboration with the IDELE, have decided to implement indoor climate diagnostics as part of the Upper Rhine Interreg project A1-9 “ResKuh.” The methodology used is the one previously developed by IDELE within the framework of the CLIMALAIT program conducted for the French Interprofessional Centre for the Dairy Economy (CNIEL). 10 farms were diagnosed during the summers of 2024 and 2025. Measurements are taken inside the building every 4 meters across both its length and width to create a complete grid of the space. The recorded data include T, RH, WS, and radiation levels obtained using a black globe thermometer. This data collection enables the mapping of the buildings, well as providing a representation of the THI and HLI within the livestock housing. The same measurements were taken outside at the beginning and end of the diagnostic process to establish an outdoor reference. Outside T and RH ranged respectively between 22.8 and 30.9 °C and 29 and 77 % and were an average 26.6 °C +/- 2.05 and 51.9% +/- 12.9. Inside T and RH ranged respectively between 21.9 and 31.1 °C and 30 and 81 % and were an average 26.2 °C +/- 2.18 and 55.2% +/- 12.2. The mean black globe T was 28.9 °C+/- 2.28, representing a 2.7 °C increase over the ambient T. The internal air velocity ranged between 0 and 1.68 m/s and was an average 0.52 m/s +/- 0.37. The average THI and HLI were 73.7 and 79.0 respectively, representing heat stress for the cows and a potential decreases in production. THI reached 79.0, corresponding to a high stress level. Regarding the HLI, the maximum observed value was 92.3, which is close to extreme stress. The standard deviation was 2.26 for the THI, compared to 6.34 for the HLI. This demonstrates that the HLI is a more suitable indicator for characterizing the heterogeneity of environmental conditions within a livestock building. The resulting maps allow the livestock advisor to identify the building’s strengths and weaknesses with the farmer to propose improvements: replacing materials, creating openings, or installing new equipment. This method supports farmers in adapting cattle farming to climate change and improves the resilience of this sector in the Upper Rhine region.

Why has progress stalled in reducing morbidity and mortality in dairy calves?

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Neonatal diarrhea and respiratory disease remain leading causes of morbidity, mortality, antimicrobial use, and economic loss in preweaned dairy calves. Despite decades of research identifying major risk factors, including inadequate passive immunity, suboptimal nutrition, and environmental stress, population-level reductions in disease have been inconsistent across production systems. This persistent gap between scientific knowledge and on-farm outcomes warrants critical examination. This presentation examines scientific and structural factors contributing to limited progress in neonatal calf health. Inconsistent disease definitions and outcome measures reduce comparability across studies, limit meaningful synthesis through meta-analysis, and obscure clear benchmarks for success. Greater standardization of disease definitions and outcomes is needed to improve interpretability and translation. Although pathogens initiate disease, control strategies often emphasize pathogen identification and antimicrobial treatment rather than strengthening host resilience and management conditions that prevent disease development. Furthermore, experimental studies frequently evaluate isolated interventions under controlled conditions, whereas commercial calf-rearing systems operate within complex biological, labor, and economic constraints that are rarely incorporated into study design. Substantial advances have been made in understanding passive immunity, nutrition, and environmental risk; however, neonatal morbidity and mortality remain largely unchanged because knowledge generation has outpaced knowledge translation. Research findings are frequently disseminated through scientific publications yet insufficiently integrated into practical, economically viable decision-making on farms. Bridging this gap requires deliberate incorporation of implementation science and qualitative approaches to better understand producer decision-making, along with stronger engagement of advisory networks such as veterinarians and nutritionists. Sustained reductions in neonatal morbidity and mortality will depend not only on further biological discovery, but on improving how evidence is defined, measured, and translated into consistent on-farm practice.

Impact of early dam–calf separation on serum ghrelin in dairy calves

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This study aimed to evaluate serum ghrelin concentrations in dairy calves managed under two different systems: (1) conventional separation from the dam immediately after birth and (2) dam–calf contact for at least 24 hours postpartum. We hypothesized that calves allowed early dam–calf contact would exhibit lower serum ghrelin concentrations than calves separated immediately after birth, reflecting improved satiety and reduced stress. We further hypothesized that these differences would persist during the first week after separation. Data were collected from eight Holstein dairy farms in Galicia, Spain. Serum samples were taken from 24 calves in the dam-calf contact system and 24 calves in the conventional system at three time points: just before separation (T1), two days after separation (T2), and seven days after separation (T3). Ghrelin concentrations were measured using a commercial ELISA kit, and statistical analyses were conducted using mixed linear models. Results revealed that calves allowed dam contact exhibited significantly lower ghrelin concentrations at all sampling times compared to those separated immediately after birth. These findings suggest that early maternal contact may reduce hunger-driven ghrelin secretion, likely due to improved satiety, reduced stress, and enhanced gut development. This study highlights the influence of early-life management on endocrine responses in dairy calves and supports the potential welfare and metabolic benefits of dam–calf contact systems. Further research is needed to explore the long-term impacts on calf development and production efficiency.

Early dynamics of immune, stress and redox biomarkers in serum and saliva of dairy calves with and without neonatal calf diarrhea

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Neonatal calf diarrhea (NCD) is one of the most common causes of morbidity in dairy calves during the first weeks of life and represents a major welfare and economic concern for the dairy industry. Adequate colostrum intake is essential for the transfer of passive immunity and for the development of early immune competence, influencing physiological responses to disease during the neonatal period. While blood biomarkers have been widely studied in calves, information on salivary biomarkers in newborn animals remains scarce, despite saliva representing a promising non-invasive matrix for health monitoring. This study aimed to characterize the early dynamics of immune, stress, and redox biomarkers in serum and saliva of newborn dairy calves during the first week of life and to compare healthy animals with calves developing NCD. Paired blood and saliva samples were collected from 20 newborn dairy calves on days 0, 1, 2, and 7 after birth. Biomarkers related to innate immunity (myeloperoxidase and calprotectin), cellular immunity (adenosine deaminase; ADA), humoral immunity (IgG and IgA), gamma-glutamyl transferase (GGT), stress (α-amylase), and redox status (ferric reducing ability; FRA) were analysed. IgG and IgA concentrations were determined by ELISA while other biomarkers were measured using an automated chemistry analyser. Calves received 3.8 ± 0.64 L of colostrum within 96 ± 73 min after birth, and health status was monitored daily using an adapted Wisconsin calf health scoring system. All biomarkers were measurable from birth in both fluids. Innate and cellular immunity biomarkers were generally present at higher concentrations in saliva than in serum, whereas humoral, stress, and redox biomarkers were higher in serum. Following colostrum intake, increases were observed in saliva calprotectin, saliva and serum ADA, serum GGT, and both serum and saliva IgG and IgA concentrations, reflecting early immune activation and transfer of passive immunity. By day 7, nine calves showed signs of NCD, with the first clinical signs appearing from day 4. Calves with NCD showed higher serum myeloperoxidase (73.8 [33.6, 162.2] UI/L) and α-amylase (182 ± 14.2 UI/L) activities than healthy calves (20.5 [10.0, 41.7] and 115.0 ± 12.9 UI/L, respectively; $P < 0.05$). In saliva, calves with NCD tended to show higher GGT ($P = 0.09$) and FRA ($P = 0.059$) activities. These findings highlight the influence of colostrum intake on early biomarker dynamics and support saliva as a promising non-invasive matrix to monitor neonatal calf health. However, as clinical signs of NCD appeared between days 4 and 7 and biomarker measurements before disease onset were limited to day 2, further studies with higher sampling frequency during the first week of life are needed to better characterize biomarker changes preceding disease development. Funding: This work is funded by National Funds through FCT – Foundation for Science and Technology under the Project UID/05183/2025.

Maternal metabolic status during late gestation of dairy cows and its association with off-spring birth weight and growth.

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Good care of calves on a dairy farm ensures that calves are healthy and grow up and develop well. Extensive knowledge is present how the farmer can manage calf health and development. Some knowledge exists on the relation between maternal physiological status and calf characteristics. For example, elevated non-esterified fatty acids (NEFA) in plasma of cows during late gestation have been associated with lower birth weight of the calf (Ling et al.,2018). It can be hypothesized that the metabolic status of dry cows is associated with offspring birth weight and postnatal growth. The aim of this study was to evaluate the relationships between metabolic characteristics of dairy cows during late-gestation and birth weight and growth of their offspring. This observational study was conducted on a Dutch dairy farm from February 2024 to June 2025. For the current study prepartum metabolic status of 173 Holstein Friesian cows was evaluated by measuring plasma non-esterified fatty acids (NEFA), β-hydroxybutyrate (BHB) and assessing body condition score (BCS, 1-5 scale) at 14 d and 7 d before expected calving date. Body weight of the calves was recorded at birth and weekly until 3 weeks of age, and postnatal growth was calculated as the average daily gain (g/d) between birth and 3 wks of age. Retrospectively, cows were classified into a low and a high class for each variable. For NEFA, cows were classified based on the median (0.15 mmol/L) into low NEFA (mean ± SD: 0.10 ± 0.02 mmol/L, n=86) and high NEFA (0.27 ± 0.15 mmol/L, n=87). For BHB, cows were classified based on the median (0.49 mmol/L) into low BHB (0.42 ± 0.06 mmol/L, n=86) and high BHB (0.59 ± 0.14 mol/L, n=87). For BCS, cows were classified based on the median (3.3) into low BCS (2.8 ± 0.29, n=81) and high BCS (3.6 ± 0.37, n=92). Body weight at birth and calf growth were analysed using a mixed model (PROC MIXED, SAS), with parity (1, 2, 3, or >3), calf breed (Holstein Friesian (HF) or Holstein Friesian × Belgian Blue cross (HFBB)), calf sex (male or female) and twin status (yes or no) and either NEFA, BHB, or BCS class (high or low) as fixed effects in three separate models. Birth weight of female calves born to cows with high NEFA was higher than that of female calves born to cows with low NEFA (40.9 kg vs 36.5 kg, $P<0.01$), while this relationship was not present in male calves. No differences in birth weight were observed among calves born to cows with different BHB or BCS classes. For all models, parity, calf breed, and calf sex ($P<0.05$) were related to birth weight. For the models with NEFA and BHB, twin status was also related to birth weight ($P<0.05$). Prepartum NEFA, BHB or BCS class were not related to offspring growth in the first 3 weeks. For all three models, an interaction between parity and calf sex ($P<0.05$) was present. In conclusion, maternal NEFA status during late gestation, but not BHB or BCS status, was associated with birth weight, and exclusively in female offspring. These findings contrast with those of Ling et al. (2018), which may be explained by differences in parity distribution in the study population or class cut-off values. In the current study maternal metabolic status was not related to growth of the calves till 3 weeks of age. Maternal metabolic status as a predictor of offspring birth weight may enable farmers and advisors to further optimise the care of both cow and calf.

Do dairy calves reared by foster cows benefit from the additional provision of milk from an automatic feeder?

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In foster cow systems, cows typically nurse several foster calves (FC) in addition to their own calf (OC). Differences in cow-calf bonding may disadvantage foster calves in terms of growth and health. The aim of this study was to investigate whether providing supplementary milk via an automatic milk feeder (AMF) could mitigate this risk. We hypothesized that (1) FC would use the AMF more than OC, and (2) differences in growth and health between OC and FC would be less pronounced in groups with AMF access. On a large commercial dairy farm, two treatments (access to AMF versus control, CON) were compared, with one repetition (April–August 2025 and July–December 2025). Out of the 29 OC and 29 FC in total, 18 pairs of OC and FC control calves and 11 pairs of OC and FC AMF calves were monitored from birth to weaning. AMF calves were trained to use the AMF during the first two weeks of life. Milk allowance started at 1 L/day (weeks 1-2), increased to 4 L/day until week 6, and was reduced gradually from week 12 until weaned (week 20). Health indicators were assessed six times per calf as absent (0) or present (1) with respect to impaired vitality, poor hair and body condition, gastrointestinal and respiratory symptoms, and they were summed to a composite health impairment score (0-8). Concurrently, the calves were weighed to derive individual daily weight gains per suckling phase, as well as weaning weights. Individual milk intake and visit frequency at the AMF were recorded automatically. Linear mixed models were used to test effects of calf status (FC vs. OC), treatment (AMF vs. CON), suckling phase (beginning of rearing, middle of rearing, pre-, and post-weaning), and interactions. After training (beginning of rearing), all calves continued visiting the AMF, yet on average only on 26 ± 16 and 29 ± 9 days (FC and OC) from a maximum of about 130 days in total. The average total milk intake was substantially lower than expected and lower than the milk allowance (FC: 3.17 L, OC: 3.15 L from potentially about 380 L) (status: p=0.979). ADWG increased with age (phase: p= 0.025). At the beginning of rearing, ADWG was comparable within FC (AMF: 0.66 kg/day, CON: 0.65 kg/day) and OC (AMF: 0.81 kg/day, CON: 0.78 kg/day). At the middle of rearing, AMF calves had numerically higher weight gains (FC: 1.09 kg/day, OC: 1.23 kg/day) than control calves (FC: 1.00 kg/day, OC: 0.99 kg/day), yet, differences in ADWG were not statistically supported for status (p=0.734), nor treatment (p=0.752). Nevertheless, preliminary results show numerically higher weaning weights in AMF calves (FC: 175 kg, OC: 182 kg) compared to controls (FC: 155 kg, OC: 152 kg). However, there was high variance in weights within all groups, especially within control FC. Health impairment scores were not affected by calf status (p=0.956) or treatment (p=0.777), but at each suckling phase, half of the calves (median) showed clinical symptoms in at least two health indicators, in particular nasal and ocular discharge. Overall, AMF use was substantially lower than anticipated. Nevertheless, calves with access to an AMF had numerically higher weaning weights. There was no evidence for FC being disadvantaged in either treatment. In general, the need to improve health management became apparent. The reasons for the low AMF use should be further investigated, including taking a closer look at calf behavior.

Progressive delay of suckling opportunities: effect on weaning distress in a cow-calf contact system

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In cow-calf contact (CCC) systems, human-induced weaning before the natural weaning age exposes calves to both physiological and social distress. In a calf-driven CCC system, in which suckling usually occurred after the partial separation due to morning and evening milking, we tested whether postponing suckling opportunities in weekly or daily steps could further alleviate distress at weaning. We hypothesized that postponing suckling opportunities daily would lead to fewer access-trial to enter the cow herd than postponing it weekly. Until the 9th week of age, calves were free to meet and to suckle their dams in the cow herd for 17 h d^{-1}. From the 10th week of age, the access time to the cow herd was postponed after morning milking by 35 min per day (Smooth, n = 10 calves) or weekly in 4-h steps (Gradual, n = 10 calves). Similarly, from the 12th week of age, the access time was postponed after evening milking. In the 14th week of age, calves were weaned and fence-line separated from their dams. Data were collected from the 9th until the 14th week of age. The time spent in the cow herd and the hourly count of calves' denied access to the cow herd were recorded daily. A statistical analysis was conducted using (generalized) linear mixed models. On a weekly basis, the time spent in the cow herd and the number of denied access to the cow herd did not differ between treatments. However, the distribution of denied accesses over time differed between treatments (3-way interaction between treatment, day and time, $P < 0.001$). On the first day that the access to the cow herd was postponed after morning milking, Gradual calves had more denied access compared with the seventh day after the initiation of the postponed access. This difference was noticeable for both the first and fourth hours following the end of the morning milking (odds ratios for the first hour of delay: 4.3, $P = 0.007$ and the fourth hour of delay: 2.3, $P = 0.011$). Among Smooth calves, during the first hour following the end of the morning milking, the number of denied accesses was similar between the first and the seventh day following the initiation of postponed access. However, during the fourth hour following the end of the morning milking, less denied accesses were recorded on the first day compared with the seventh day (odds ratio: 0.002, $P < 0.001$). In both treatments, the pattern of access-trial following the initiation of postponed access after evening milking was similar to that observed when the access was postponed after morning milking. The decline in the number of denied access over the days following the initiation of postponed access suggests that Gradual calves did not attempt to join the cow herd continuously and might have learned a new entry time. However, the number of denied access of Smooth calves increased during the later hours of delay over the days. Smooth calves might have continued attempting to access the cow herd until access was allowed. Because of the experimental design, Smooth calves must wait every day for a longer time before entering the cow herd and they might lack a consistent reference entry time. On a weekly basis, the absence of difference between treatments in the number of denied access to the cow herd indicates that postponing suckling opportunities with daily steps did not reduce the number of denied access to the cow herd when compared with weekly steps. To conclude, postponing suckling opportunities with daily steps did not further reduce calves' motivation to enter the cow herd during weaning.

Feasibility of using a mobile platform application for farmers to submit observations of Maternal Protective Behaviour in Cow-calf contact herds.

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With the growing interest in cow-calf contact (CCC) systems in dairy production, novel methods to research on-farm CCC events are relevant. A mobile platform application may facilitate farmer self-reports of CCC-related variables but its feasibility (e.g validity) need to be tested. The objective of this experimental study was to evaluate the feasibility of using a mobile platform application for farmers to submit observations of Maternal Protective Behaviour (MBP). More specifically to study the proportion of cows calving in a herd during the study period for which MPB was scored and registered by the farmer. Since Norwegian farmers are already accustomed to the use of mobile platform applications to submit dairy herd relevant data, we hypothesized that the use of another application would be feasible for farmers. A cohort study was initiated with the following inclusion criteria for cohort farms: commercial dairy herd milk delivery to TINE Dairies, cow breed (Norwegian Red), main CCC system (dam-calf contact), milking cows in an automatic milking system (AMS), willingness to share data from the national dairy herd recording system and farmers having time to submit registrations. Ten farms were included in the study. For all cows calving within an 18-month study period, farmers were instructed to submit self-reported MBP assessed 0-48 hours after parturition by observation at two contexts: in the calving pen (undisturbed) and when the cow was fixated in the maternity pen for milking and the calf handled (fixated). To evaluate the feasibility of using a mobile platform application for farmers to submit observations of MPB, we divided the herd number of cows for which MPB was registered in the study period by the herds total number of calvings recorded in the National Dairy Herd Recording System during the same period. The mean (±SD) herd number of observations was 43 (25). One herd submitted zero registrations, and one herd submitted two registrations. Mean herd number of calvings per herd was 67 (21). The mean herd proportion of calvings for which MBP was registered in the mobile application was 67 (37) %, with two herds submitting 0 or only ≈ 2%. The feasibility of using a mobile platform application for farmers to submit observations of MBP appears high for most farms, but two farms submitted no or very few registrations despite receiving close follow-up. The feasibility of using the mobile platform application appears to be relatively high but also farmer-dependent. This work was co-funded by the European Union's Horizon Europe Project 101136346 EUPAHW. Views and opinions expressed are those of the author(s) only and do not necessarily reflect those of the European Union or the European Research Executive Agency. Neither the European Union nor the granting authority can be held responsible for them.

Milk yield and composition changes in cows following short-term separation after one-week of cow-calf contact compared with early separated cows

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Cow–calf contact provides benefits for calf development and welfare, but short or long-term separation often leads to alterations in cows performance and metabolic parameters. The aim of this study was to investigate the effects of separation after one-week cow-calf contact (CCC group) on milk yield and composition and to compare these outcomes with those of early-separated cows (ESG group). The study was conducted at the experimental farm Veitshof of the Technical University Munich in Freising-Weihenstephan. Twenty clinically healthy cows without dystocia were enrolled, blocked by parity, and randomly assigned to either the CCC group (n = 10) or the ESG group (n = 10). All cows received a partially mixed ration formulated to meet their nutritional requirements. In the ESG group, calves were separated within ≤ 2 hours (h) after birth and housed individually in calf igloos, while cows remained in the calving box for health monitoring before reintegration into the lactating herd, at the same postpartum (p.p.) time point as CCC cows (day 8 p.p.). Calves in the CCC group had unrestricted access to their dams except during twice-daily milking (< 1 h/day). Milk yield was recorded at each milking using a calibrated floor scale. Milk samples from days 4-10 p.p. were analyzed for dry matter and ash content, and for milk components. Fat, protein, lactose, urea, and pH were determined using a MilkoScan 7 RM (FOSS, Denmark), and somatic cell count was measured using a Fossomatic 7 (FOSS, Denmark). A linear mixed-effects model was applied with time, group, and the group × time interaction as fixed effects, and cow as a random intercept (~1 | cow). Across the first week, collected milk yield was higher in ESG cows than in CCC cows, with an average difference of 12.86 ± 1.04 L/day between days 1 and 7 p.p., converging only around days 9-10. Following separation on day 8, CCC cows displayed metabolic stress indicators, such as higher milk fat, an elevated fat-to-protein ratio, and increased milk urea, indicating a transient negative energy balance and short-term risk of ketosis. Lactose concentrations were lower in the CCC group until day 8, but increased thereafter to levels comparable to those of the ESG group. The somatic cell count remained largely comparable between groups, except for an isolated increase on day 7 in CCC cows. Overall, milk yield and composition patterns indicated that lower measured yields under pre-separation CCC conditions were attributable to milk removal by calves rather than reduced milk synthesis, and that targeted energy supply and stress mitigation following separation are critical for maintaining post-separation performance and cow health.

First-lactation milk yield and growth performance of dairy heifers reared with prolonged dam-calf contact

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In artificially reared calves, greater preweaning growth has been associated with higher milk yield during the first lactation. Dairy calves that are instead reared with prolonged dam-calf contact demonstrate high growth rates during the preweaning period, likely due to consuming large volumes of milk. However, the long-term effects of dam rearing on later productive performance remain unclear. This study used a retrospective cohort study design to (1) explore milk yield of dam-reared heifers during their first lactation, and (2) evaluate the effects of preweaning management (artificial vs dam rearing) on average daily gain (ADG) before and after weaning. A total of 61 heifers reared with 3–6 months of dam-calf contact (dam-reared; DR) were matched 1:1 to artificially-reared heifers (AR; 221 candidates) using propensity scores. Only animals that had calved at least once were eligible for matching. Preweaning management differed between cohorts: DR heifers were raised with whole-day dam-calf contact across 5 experimental trials, whereas AR heifers were individually and/or group-housed and fed 6–9 L/d of whole milk until weaning at 2.5 months. After weaning, all heifers were managed the same way. Body weight data was used to calculate ADG during the pre- (birth to weaning) and postweaning (weaning to first insemination) periods. Linear mixed-effects models were run to test for differences in ADG between cohorts, while exploratory linear models were used to assess daily and 305-d energy-corrected milk yield (ECM; kg) as well as lactation persistency (Δ kg/d between 100 and 250 days in milk). Importantly, the study was underpowered for milk yield outcomes; however, the scarcity of long-term research justifies exploratory analyses. Results showed that DR heifers grew at higher rates until weaning (1.3 vs 0.9 kg/d for AR heifers; $P < 0.001$) but experienced growth checks during the subsequent 2 weeks, which were not observed in the AR cohort. During the postweaning period, this pattern was reversed (DR: 0.8 kg/d, AR: 0.9 kg/d; $P = 0.005$), although DR heifers were numerically heavier both at first insemination and immediately after first calving. Differences in milk yield were also evident, with DR primiparous cows showing lower 305-d ECM yield ([estimated marginal mean] DR: 8957 kg, AR: 9455 kg; 95% CI for DR: −987 to −8 kg), daily ECM yield (DR: 29.8 kg/d, AR: 31.7 kg/d; 95% CI: −3.6 to −0.1 kg/d) and persistency (DR: −0.033 kg/d, AR: −0.014 kg/d; 95% CI: −0.035 to −0.005 kg/d). Quartile-level analyses revealed that 305-d yield was numerically similar among the 25% highest-yielding cows within each cohort (mean [SD]; DR: 10789 [694] kg, AR: 10870 [708] kg), whereas differences increased progressively toward the bottom quartile interval (25% lowest; DR: 6620 [661] kg, AR: 8033 [730] kg). Interestingly, 9 of the 13 lowest-yielding DR cows were reared within the same experimental trial. While this study provides preliminary evidence of potential milk yield losses associated with prolonged dam-calf contact (i.e., 3–6 months), at least in some animals, further longitudinal research is needed to evaluate how different dam-rearing management strategies influence later productive performance.

Two paths to one estimate: Cow- and calf-derived methods to quantify milk production in full cow-calf contact systems

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Dairy cows in full cow-calf contact systems can nurse their calves and perform maternal behaviours throughout the day. However, quantifying total daily milk production of suckled cows is challenging, as machine-recorded milk yield excludes the milk consumed by the calf. We aimed to determine total daily milk production of dairy cows with full cow-calf contact during the suckling period, using two methods based on cow and calf performance data. Dairy cows at the Swedish Livestock Research Centre either had full contact (FC, n = 18) with their 19 calves until gradual fence-line weaning at 13.0 ± 1.1 wk of age, or were conventionally managed with no contact (NC, n = 20) with their 22 calves beyond 13.5 h after birth. Individual daily milk yield to the automatic milking unit and cow roughage and concentrate intake were recorded throughout. Daily milk production of the cows during the FC suckling period was estimated from recorded milk yield and cow energy intake data (Method 1) or from recorded milk yield and calf performance data (Method 2). Method 1: Individual milk production was calculated as the product of daily energy intake during suckling and the median ratio of recorded milk yield to previous-day energy intake from the 8-wk post-suckling period. Recorded milk yield and estimated milk production of all cows were analysed with linear mixed models. Estimated FC milk production averaged 35.3 ± 1.40 kg/d during the suckling period, first 12 wk of lactation, while their recorded machine milk yield averaged 24.7 ± 1.60 kg/d. Estimated NC milk production averaged 33.3 ± 1.40 kg/d (not different from FC; P = 0.29) during the first 12 wk of lactation, while their recorded machine milk yield averaged 37.7 ± 1.40 kg/d (greater than FC; P < 0.001). This discrepancy between recorded and estimated milk yield of NC cows may reflect unaccounted negative energy balance likely experienced. Method 2: Individual milk production was calculated as the sum of daily recorded milk yield and estimated calf milk consumption. Calf milk consumption was estimated by subtracting concentrate-derived energy from total energy requirements of the calves for maintenance and growth, subsequently dividing this residual energy by the calculated milk energy content. Estimated FC cow milk production and recorded NC milk yield were analysed with a linear mixed model. Average FC calf milk consumption during the suckling period was estimated at 14.9 ± 2.9 kg/d, whereby FC cow milk production was estimated at 37.9 ± 1.80 kg/d during the suckling period of the first 12 wk of lactation (not different from NC recorded yield; P ≥ 0.59). In conclusion, using energy intake and milk yield during the non-suckling period underestimated milk production of FC cows during the suckling period. In contrast, estimated milk production of suckled FC cows, based on their calves' estimated milk consumption, did not differ from milk production of NC cows based on recorded machine milk yield. Although calf milk consumption could not be measured directly, the evaluated methods for estimating total milk production of suckled cows and calf milk consumption can be applied in economic and environmental sustainability analyses of cow-calf contact systems.

Long-term calf nursing compared to conventional milking affects milk yield and milk fat content after calf weaning

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The separation of cows and calves in dairy production is increasingly criticized due to animal welfare concerns. Alternative systems allowing prolonged cow-calf contact are gaining interest, yet previous studies have yielded inconsistent results regarding milk yield and composition. Since dam-calf contact often leads to milk ejection issues during milking, foster cow systems might serve as a compromise between the animals´ and farmers´ needs. After calf weaning, we investigated the differences in milk yield and milk composition between cows that had nursed 3-4 calves for 9- 16 weeks, and cows that had been separated from their calf within 24 h post partum and milked twice daily afterwards. We expected former foster cows to exhibit milk ejection problems after being introduced to the milking herd on the short-term, but to show a similar milk yield and composition after habituation to being milked. On a large dairy farm, a total of 21 foster and 21 conventionally milked cows were matched according to breed, parity, age, and calving date. Data collection included performance parameters (milk yield, fat content) from the regular monthly milk recordings throughout the milking period after calf weaning until 400 DIM. 10 cows left the herd due to illness during data collection and were not present until the end. After weaning, all cows were housed in cubicle stalls. The effect of cow status (foster or milked), days in milk (DIM), and its square, along with their interactions on milk yield and fat content were analyzed using linear mixed models. The animal identity nested within the pair, was included as random effect. After separation from the calves (DIM 60-134), former foster cows started with a lower milk yield than conventionally milked cows, but the two groups converged over the course of lactation (status × DIM: p=0,001), and we found only slight differences for total yield after calf weaning (foster = 28.3 kg/day, milked = 29.3 kg/day, p=0.24). Former foster cows showed a slight increase in fat content toward the end of lactation. However, this difference could not be statistically supported (status x DIM: p=0.26, status x DIM^2: p=0.13). The lower milk yield in foster cows after separation from the calves was likely attributed to either a lower milk production during the suckling period or to a reduced oxytocin release during milking, resulting in impaired milk ejection. Milk ejection problems may lead to incomplete emptying of the udder, resulting in an increased amount of residual milk. This could increase the risk for mastitis, which needs to be investigated in CCC systems. We had expected fat content to be reduced in foster cows due to impaired milk ejection, but this was not the case. Altogether, cows that had nursed their own and 2-3 foster calves over a period of several weeks were found to show only a temporarily reduced milk yield and no difference in milk fat content in the milking parlour compared to conventionally milked cows on the same farm.

Effect of cow-calf contact on milk yield and milk flow in an automatic milking system before, during and after separation from the calf

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Interest in cow-calf contact (CCC) dairy systems is increasing. One of the main challenges is lower yield of saleable milk both during and after separation from the calf. This is particularly problematic for farms that do not sell their milk with added value. It is important to clarify the mechanisms behind the lower milk yield and the aim of this study was to increase the understanding of links between milking characteristics and milk yield. A total of 38 cows (26 Swedish Red, 12 Swedish Holstein) calving in October and November 2023 were blocked according to breed and calving date and assigned to Conventional (CONV) or CCC dairy management in a voluntary milking system (DeLaval VMS, DeLaval International AB, Tumba, Sweden). CONV and CCC cows were housed in the same VMS, but CCC had access to a calf contact area that was unavailable for CONV cows. CCC cows could access the contact area at any time of the day and stay as long as they wanted for the first 85±16 days of the lactation (phase 1). After that a fence-line weaning protocol was applied, that allowed cows and calves to touch each other and also allowed stepwise decreased opportunity to suckle through a gate for two weeks (phase 2), until they were completely separated (phase 3). Udder quarter level data on peak and average milk flow (ml/min) and milk yield per milking and per day was extracted from the DelPro system (DeLaval International AB, Tumba, Sweden) for four days during each phase. Data is shown as mean ± SD or as coefficient of variation (CV) in percent. Daily milk yield in CONV was 41±12, 39±11 and 40±10 l/day in phase 1, 2 and 3. Milk volume extracted when milking the CCC cows was 26±13, 35±12 and 37±12 l/day during the same period, indicating that total milk yield most likely was not higher in CCC cows during the contact time, or that total milk yield decreased when suckling stopped. Difficulties with milk ejection during machine milking have been proposed as a potential reason for lower milk yield post separation in CCC cows, but the data reported here does not support that suggestion. Average quarter level milk flow in CONV cows was 1058±287, 1072±280 and 1072±291 ml/min during phase 1, 2 and 3 while it increased in CCC cows from 1073±378 to 1134±304 and 1215±364 ml/min. Peak flows were 1503±349, 1514±338 and 1520±361 in CONV and 1546±468, 1612±369 and 1697±439 in CCC cows, which indicates higher, and gradually increasing, peak flows in CCC cows. Variation was larger for both peak and average flow in CCC (CV 27 and 31% for peak and average flow) than in CONV cows (CV 23 vs 27% for peak and average flow), particularly in phase 1 (peak flow: 23 vs. 31% and average flow: 27 vs 35% for CONV vs CCC). If the individual variation in milk flow is constant across CCC lactations, it opens up for selective breeding favoring cows that have the ability to eject milk to the milking machine in parallel with interacting with calves in CCC systems.

Evaluation of bioactive core based on plant extracts to support neonatal calves diarrhea

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Neonatal calves diarrhea is a major issue in livestock farming, with early-life infections causing long-term impacts on health and growth. During the vulnerability period, when calves shift from colostrum-derived passive immunity to their own active immunity, the gut barrier remains immature, prone to oxidative stress, and highly sensitive to enteropathogenic bacteria (e.g., Escherichia coli, Salmonella spp., Clostridium perfringens). This study focused on early gut health, by investigating the effects of different plant extracts (PE) for inclusion in a milk-dispersible formulation designed to support calves health through their antioxidant properties and their ability to limit the growth of diarrheagenic bacteria. Six PE including ceylan cinnamon (CEY); guava (GUA); clove (CLO); chestnut tannin (CHE); oregano (ORE); and thyme (THY) were included in this project. First step was to test PE in vitro against 6 pathogens involved in calves diarrhea (E. coli, S. typhimurium, S. dublin, Y. enterocolitica, C. jejuni and C. perfringens), using a spot assay, at two different concentrations (1 g/L and 5 g/L). For each pathogen, control (no PE) and copper sulfate negative control were included and all conditions were tested in triplicate. Second step was to evaluate every PE antioxidant capacity using 3 complementary colorimetric methods (ABTS, DPPH and FRAP). Based on those primary results, the most relevant PE were combined. Three associations were developed, and the same protocol of microbiology was performed (the same 6 major pathogens tested) to confirm final in vitro effects. Microbial data were analyzed using two-way ANOVA (PE, concentrations), whereas antioxidant measurements were analyzed using one-way ANOVA after log10 transformation to correct skewness and heteroscedasticity, followed by Tukey's post-hoc test using R software. First spot assay on the 6 PE showed that 4 of them (CEY, CHE, CLO and GUA) had significant inhibition rate on at least 3 pathogens ($p < 0.05$). We observed a complete inhibition of S. typhimurium growth with CEY; CLO and CHE; of Y. enterocolitica with CHE and of C. jejuni with CEY; CLO; GUA and CHE. Across the 3 antioxidant assays, CHE showed antioxidant activity 2 to 10 times greater than all other PE, with all differences being significant ($p < 0.01$). These primary results led to three combinations formulated around one major PE: CHE-based; GUA-based and CEY-based formulas. Antimicrobial properties tested on the three associations showed that at the highest concentration, all of them had significant inhibition effect on the same 3 pathogens (S. typhimurium; C. jejuni; C. perfringens). At the lowest concentration, only CHE-based formulation had significant inhibition activity on 2 pathogens (S. typhimurium and C. jejuni). The in vitro trials showed that the CHE-based combination had significant effect on the antioxidant capacities and antimicrobial properties, which are main risks factor of diarrhea. This promising formula is going to be tested on meat and dairy farms, diluted in whole milk to investigate the in vivo efficiency on diarrhea criteria (occurrence, severity, duration) while maintaining optimal milk consumption and growth performances.

Alterations in milk composition and fatty acid profiles as indicators of subclinical and clinical mastitis in Holstein cows

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Mastitis remains one of the most significant challenges for animal welfare, while early detection of subclinical mastitis (SCM) is crucial for minimizing production losses. The inflammatory process leads to the impairment of the blood-milk barrier integrity and a subsequent suppression of the secretory activity of mammary epithelial cells. This study aimed to evaluate changes in lactose, citric acid, and milk fatty acid profiles in Holstein cows in relation to their SCM and clinical mastitis (CM) status. A robust dataset of 11,757 milk performance recordings (4 herds, 5–305 days in milk) was analysed, where SCM was defined as a somatic cell count (SCC) > 200,000 cells/mL of milk and CM was identified based on veterinary diagnosis. Statistical analyses were performed using the SAS package (ver. 9.4). The data were analysed using a generalized linear model to compare least squares means, with the effects of herd, year, season, test-day order, breed, and parity being taken into account. Results showed a prevalence of 22% for SCM and 6% for CM. In comparison with healthy individuals, SCM cows exhibited significantly lower lactose (4.83% vs. 4.95%, respectively; $P < 0.001$) and citric acid levels (6.42 mmol/L vs. 6.53 mmol/L, respectively; $P < 0.01$). A moderate negative relationship was demonstrated between the lactose content and the somatic cell score ($r = -0.40$). The reduction in lactose and citrate levels in SCM cows reflects the impaired secretory activity of lactocytes and increased permeability of paracellular pathways. Regarding the fatty acid profile, SCM resulted in a significant inhibition of de novo synthesis. This was evidenced by the lower proportions of short-chain fatty acids and C14:0 in SCM cows compared to those in healthy cows (12.44 vs. 12.78 g/100 g of milk fat and 10.271 vs. 10.45 g/100 g of milk fat, respectively; $P < 0.001$). Conversely, an increase in preformed fatty acids was observed in SCM cows, particularly C18:1 (28.25 vs. 27.91 g/100 g of milk fat; $P = 0.001$) and C18:0 (8.46 vs. 8.39 g/100 g of milk fat, respectively; $P < 0.001$). These findings support the hypothesis of disrupted lipid metabolism in the early stages of inflammation. Reduced de novo fatty acid synthesis is an indicator of the inflammatory process before clinical signs become apparent. As was the case with SCM cows, lower levels of lactose were found in CM cows compared to healthy cows ($P < 0.001$), while the difference in citrate was not significant. In conclusion, these metabolism-induced shifts in milk represent sensitive biomarkers of impaired udder functional integrity, offering potential for automated on-farm health monitoring systems. The study was funded by the Ministry of Agriculture of the Czech Republic (NAZV QL24020269).

Relationships between feeding behaviour and milk fatty acid profiles in lameness-affected Holstein cows

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Lameness is one of the most prevalent health problems on dairy farms, with a significant impact on both animal welfare and farm economics. It is widely acknowledged that early identification of affected animals is essential to minimize production losses. The objective of this study was to quantify alterations in feeding behaviour and detailed milk composition associated with lameness in Holstein cows. The investigation utilised a robust dataset, which comprised 9,134 records. The animals were categorised as either healthy or diseased based on clinical diagnoses made during the month of milk recording. Milk composition and fatty acid profile analysis were performed via mid-infrared (MIR) spectroscopy exclusively on samples collected during afternoon milking. Statistical analyses were performed using the SAS package (ver. 9.4). The data were analysed using a generalized linear model to compare least squares means , with the effects of herd, year, season, test-day order, breed, and parity being taken into account. The results demonstrated that lameness-affected cows exhibited a highly significant deficit in feeding behaviour compared to healthy individuals, characterised by a reduction in daily feeding time of 44 minutes (295 vs. 340 min, respectively; $P < 0.001$). In contrast, the analysis of rumination time showed only a marginal decease of mere 8 minutes (447 vs. 455 min, respectively; $P < 0.05$), indicating that the assessment of time spent at the feeder is a more sensitive indicator of discomfort associated with painful standing. This behavioural shift resulted in a significant drop in afternoon milk yield by 0.7 kg (15.61 vs. 16.32 kg, respectively; $P < 0.001$). Detailed analysis of the milk fatty acid profile revealed a specific metabolic response to reduced nutrient intake and stress. The proportion of preformed fatty acids was found to increase significantly in the diseased compared to healthy cows, particularly C18:1 (28.61 vs. 27.31 g/100 g of milk fat, respectively; $P < 0.001$) and C18:0 (8.83 vs. 8.48 g/100 g of milk fat, respectively; $P < 0.001$), indicating mobilization of body reserves. Conversely, a significant decrease was observed in medium-chain fatty acids, specifically C14:0 (10.05 vs. 10.51 g/100 g of milk fat, respectively; $P < 0.001$) and C16:0 (26.45 vs. 27.26 g/100 g of milk fat, respectively; $P < 0.001$), while the proportion of short-chain fatty acids remained unaffected. The milk lactose content demonstrated notable stability (4.91 vs. 4.92%, respectively; $P = 0.09$), confirming that the observed changes in milk fat composition are a consequence of energy deficit and behavioural adaptation rather than disruption of the mammary secretory epithelium. The integration of sensor-derived behavioural data with MIR-based milk profiling has been identified as a potential tool for precision health management in commercial dairy herds. The study was funded by the Ministry of Agriculture of the Czech Republic (NAZV QL24020269).

Mucin distribution and remodelling along the bovine gut-lung axis during respiratory infection

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Mucins are a critical component of mucosal defence, yet their distribution and responsiveness to respiratory pathogens remain poorly characterized in calves. To address this gap, we examined 28 animals, half experimentally infected with Mycoplasma bovis, influenza D virus, and bovine coronavirus, and monitored them until necropsy at 21 days post-infection. Combined Alcian Blue (AB) and Periodic Acid-Schiff (PAS) staining was used to quantify acidic and neutral mucins along the respiratory and digestive tracts, and to correlate these patterns with pathogen burden and lesion severity. Mucin architecture showed pronounced anatomical segregation: the large intestine displayed a dense, acidic, mucin-rich barrier, whereas respiratory lower tract exhibited uniformly sparse, low mucin coverage. Despite clear evidence of infection and associated pulmonary lesions, lung mucin abundance showed minimal remodelling and did not correlate with pathogen load, except for a modest negative association between neutral mucins and M. bovis in the caudal lobe. In contrast, the histological surface of intestinal neutral mucins increased in parallel with respiratory pathogen burden, revealing divergent, organ-specific mucin–pathogen relationships along the gut–lung axis. Together, these findings suggest that mucins in young calves' function as relatively static structural features in the lungs but as more dynamic components of the intestinal barrier. This histopathological characterization will guide upcoming immunohistochemical and RNA-based analyses targeting specific mucins to clarify their roles in mucosal defence and their potential relevance to bovine respiratory disease.

Impact of postnatal drying management and ambient temperature on microbial colonization in neonatal calves

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Evaporation of amniotic fluid exposes newborn calves to a cooling effect and may influence calves' lying time after birth, attempts to stand up, colostrum intake and thus affect energy status, passive immune system and first contact with pathogenic microorganisms. Licking by the cow may stimulate the calf´s vitality and accelerate drying. A dry hair coat forms a protective barrier against both cold and pathogens. The aim of this study was to investigate the effects of different drying methods in early separated dairy calves on the microbial colonization of the calf´s hair coat, with particular consideration of the drying process, rectal temperature and climate conditions. Newborn Brown Swiss calves (n = 16) were randomly assigned to one of three drying treatments: one hour maternal licking (ML), rubbing with straw (SR), and covering with a waterproof calf blanket without prior drying (BC). Sampling and data recording were conducted four times: immediately after birth (0h) and at one, four, and twelve hours postpartum. Additionally, at each sampling time, the drying level of different body regions (head, peripheral ear, neck, nape, shoulder, back, belly, foreleg, hind leg, hip, loin, tail) was scored (4: dripping wet; 3: like a wrung out towel; 2: slightly damp; 1: completely dry), and rectal temperature, body surface temperature, relative humidity and ambient temperature in the calving area were recorded. Calf vitality, health status, and the calving conditions were also documented. Microbial surface samples per calf were collected using a standardized sampling technique with a sterile cotton glove, which was transferred to a sterile saline solution. Microbiological analysis was performed by culturing total bacterial count (TBC), Enterobacteriaceae, Coliforms, Lactobacilli, and Staphylococci. All calves dried over time ($p<0.05$). Except for rapidly drying areas, significant differences in drying level were observed between the treatment groups, whereby body regions covered by a blanket remaining wet for longer compared to the same regions of calves dried with straw or by maternal licking. At the belly and hind limb, calves that were licked by the dam were drier than those in SR ($p<0.05$). The temperature humidity index (THI) showed an influence only on the peripheral ear and highly mobile (neck, belly, lateral hindquarter) body regions ($P<0.05$). Rectal temperature as well as body surface temperature did not differ between treatments but were influenced by sampling time, ambient temperature and THI ($P<0.05$). Bacterial colonization at birth was mainly influenced by dystocia and associated human handling of the calf ($P<0.05$). The drying method had hardly any effect on bacterial quantities, however Enterobacteriaceae, coliform bacteria, and lactobacilli changed over time ($p<0.05$). In ML calves, Enterobacteriaceae and Coliforms decreased over time, whereas they remained relatively constant in calves dried with straw or covered with a blanket. TBC and Staphylococci decreased significantly with increasing THI. Waterproof blankets impede the evaporation of amniotic fluid and may lead to prolonged wetness and increased potential for delayed cold stress. Additionally, the hair coat dries in a flattened position, which may impair its insulating function. It seems important to dry calves before applying a calf blanket. Microbial results point to the importance of hygiene during calving.

Breeding broilers against growth-related 'defects' – lessons from the past to the future

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Since the 1940s, higher growth rate, being essential for efficient meat production, has been the main objective of the commercial broiler breeding programs. Due to its high heritability and ease of phenotyping, breeding for more rapid growth has been very successful, with broilers growing faster every year, continuously reaching higher body weights at earlier ages. Consequently, over the years several growth-related 'defects' have emerged, most notably (1) excessive fat deposition, (2) leg problems, and (3) ascites. Due to their association with elevated growth rate, initially each defect was considered a biological limit to selection for further higher growth rate, and therefore growth-reducing diets had been suggested to mitigate these defects, at the cost of lower overall efficiency of production. However, our experimental-selection studies, conducted since 1980, showed that the tendencies to develop these defects, although quite heritable, have been genetically independent of growth rate. Thus, identification of the genotypes prone to develop each defect facilitated selection against them while continuing successful selection for more rapid growth rate, and for other important traits (meat yield, feed efficiency, etc.). In recent years, new defects, breast muscle myopathies, have emerged: White Striping (WS, in 2007), Wooden Breast (WB, 2011), and Spaghetti Meat (SM, 2015). As with the earlier growth-related defects, the use of growth-reducing management (lower-density diets) and slow-growing breeds have been suggested to reduce the prevalence and severity (P&S) of the breast muscle myopathies, while breeding companies have amended their programs with selection against them. Indeed, WS had gone and WB is currently found only among males reared rapidly to high BW (>3.5 kg). The P&S of SM have not been declining yet, apparently due to later emergence and to different biological nature. Whereas the P&S of WB clearly increase with higher body weight and breast yield and therefore rarely found in females, the P&S of SM are higher in females than in males, and within-sex they are lowly associated with BW and breast size. Moreover, the phenotyping of SM is very difficult because this myopathy cannot be measured or assessed on live broilers, and the levels of SM observed in processed carcasses reflect the impacts of the defeathering machines, which vary considerably between days, and even between hours in a day, within each processing plant. The positive side of these effect – it is suggested that aggressive defeathering can be used as a tool to better phenotype the levels of the inherent tendency of broilers to develop SM. These phenotypic values, if heritable, are expected to lead to a successful genetic reduction in the P&S of SM. The continuous genetic elevation in growth rate and BW of broilers has been accompanied also with higher levels of internal heat. Hot ambience hinders the dissipation of this excessive heat, forcing broilers from fast-growing stocks to minimize feed intake and consequently reduce actual growth and efficiency. The susceptibility of broilers to high ambient temperatures, which increases as their daily feed intake and body weight gain increase, is a basic biological phenomenon observed in all broilers of similar growth rate and body weight. Therefore the heat susceptibility should not be considered as a heritable 'defect' independent of growth rate. Currently, the losses due to high ambient temperatures are successfully avoided (or at least reduced) by sophisticated cooling of commercial broiler houses. However, due to the high costs of artificial cooling systems and the energy they use, genetic mitigation by reducing or elimination feather coverage is suggested as sustainable future mitigation.

Genome engineering approaches to address male chick culling and broiler reproductive constraints

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Over decades of intensive genetic selection, poultry breeding has produced two highly specialized systems: layers optimized for egg production and broilers for rapid growth and meat yield. These advances made poultry one of the most efficient animal production systems worldwide, but also created biological, welfare, and economic challenges. In the egg industry, male chicks are no longer suitable for meat production, and approximately 7 billion are culled globally each year. In broiler production, selection for rapid growth has introduced physiological constraints on reproduction, requiring intensive management with welfare and economic consequences. To address these challenges, in collaboration with NextHen, we developed a genetic engineering platform enabling precise manipulation of the chicken genome and generation of transgenic lines. Using this platform, we initiated two projects to provide practical solutions. Despite different challenges, both follow three principles: (1) improved animal welfare, (2) a non-GMO end product identical to current commercial chickens, and (3) clear industry economic benefit. The first solution is the GOLDA chicken breed, developed to eliminate manual sorting and male chick culling while integrating into existing hatchery workflows. The system is based on a sex-linked construct integrated into the maternal Z chromosome. When activated, it halts embryonic development selectively in male embryos while allowing normal development of wild-type females. Activation is controlled by an optogenetic switch triggered by blue light illumination through the eggshell prior to incubation. In validation experiments, fertilized eggs from GOLDA hens were illuminated and hatched. More than 1,000 female chicks were produced with no male offspring observed, demonstrating the potential for 100% accuracy in commercial settings. The resulting females matured normally and produced eggs comparable to commercial layers. The second project, Layers Laying Broilers (LLB), addresses the conflict between rapid growth and reproductive efficiency in broiler breeding systems. The concept relies on the separation between germ cells and somatic tissues. In the LLB design, chickens possess a layer-type body (e.g., Leghorn), while their gametes carry non-GMO broiler genetics and produce broiler offspring. To control this system, a genetic model is currently being developed to eliminate endogenous layer PGCs in LLB chickens. This is based on a binary conditional design in which two breeding lines each carry one component of a sterility system. Individually fertile, their cross produces sterile offspring. Early proof-of-concept experiments demonstrate the feasibility of this approach. The LLB strategy aims to establish layer-type breeding flocks, operated under layer management conditions, that produce WT broiler hatching eggs, while addressing key animal welfare challenges in broiler breeders, including feed restriction, and enabling egg production at rates comparable to layer systems. Together, these approaches demonstrate the potential of precision genome engineering to address key structural challenges in poultry production and improving animal welfare and industry sustainability.

Broiler breeders can be selected in free-mating systems. Analysis of performance, egg quality and welfare in three breeds.

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Europe is committed to the progressive abolition of cage systems for farm animals. This shift requires adapting the reproductive methods used in broiler breeder selection, which traditionally ensure parentage control by artificially inseminating animals housed in individual cages. Transitioning to floor-based free mating systems would align with societal expectations, yet raises major questions regarding reproductive efficiency, animal welfare and health status. Scientific data on floor reproduction remain scarce, particularly for males and in fast-growing broiler breeders under feed restriction. This study aims to evaluate the feasibility of free reproduction across three broiler breeds with contrasted growth— pHu+ (≈ 61 g/d), D+ (≈ 36 g/d), X33 (≈ 26 g/d) — by evaluating their performance, health and welfare in this system. Two successive batches were reared from 18 to 33-36 weeks of age. Birds were housed on wood shaving in enriched pens (perches, nests, pecking devices) under feed restriction adapted to their breed. Initially separated by sex, birds were mixed during 3 to 6 weeks for a free mating reproduction period. Production traits included laying rate, egg quality (weight, shape index, shell thickness, shell percentage, rigidity, and breaking strength). Between 200 and 250 eggs per group were incubated to assess fertility. Welfare and health indicators comprised growth trajectory, feather condition (3-level score), and footpad dermatitis (5-level score). Statistical analyses used type II ANOVA for continuous traits and cumulative link models for ordinal scores, to assess the effects of breed, batch, age and sex on performance, health and welfare traits. Egg fertility remained consistently high across breeds (D+: 97.8%, pHu+: 95.4%, X33: 91.2%) and batches, demonstrating that floor mating ensure fertility. Laying dynamics and egg quality differed among breeds. pHu+ hens produced rounder eggs with thicker and heavier shells, and greater rigidity and breaking strength ($p<0.001$), along with better stability of shell characteristics over time compared with D+ and X33 breeds. We observed deviations in growth from theoretical cage-based curved suggesting increased energy expenditure under floor conditions, and highlighting the need to adapt feeding strategies when shifting systems. Footpad health was satisfactory, with no severe lesions recorded; however, the fast-growing breed showed greater susceptibility under higher litter moisture conditions. Feather condition declined with age, likely reflecting mating activity rather than severe aggression, which remained limited under adapted light management. Taken together, these findings demonstrate that floor-based reproduction in broiler breeders is feasible without compromising fertility. Nevertheless, genetic background strongly modulated egg quality and sensitivity to environmental conditions, with the fast-growing breed exhibiting better shell quality but greater vulnerability to footpad dermatitis. These results support the need to develop breed-specific nutritional, environmental, and genetic selection strategies for a successful transition to cage-free reproduction that preserves animal health, welfare, reproductive efficiency, and long-term breeding objectives.

Beyond additivity: SNP matrix transformation and deep learning improve genomic prediction of egg performance and quality traits in laying hens

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Accurate genomic prediction depends not only on genetic effects but also on how genomic information is represented and modeled. The genomic best linear unbiased prediction (GBLUP) partitions additive and dominance effects into separate components, assuming independence between them. This limits its ability to capture complex genomic interactions unless they are explicitly modeled. In this study, we applied two SNP matrix transformations: (i) the 2NP matrix, which concatenates the additive-centered (Z) and dominance-deviation (W) matrices into a unified input, and (ii) a locus-specific dominance-transformed matrix. Both approaches were designed to enhance nonlinear learning of genomic effects using tree-based boosting and deep-learning (DL) methods. We evaluated these transformations in combination with convolutional neural networks (CNN2NP, CNN2NPSTR, and CNNTrans) and Light Gradient Boosting Machine (2NPLGBM and LGBMTrans) using data from a commercial laying hen line. The predictive performance of these models was compared with GBLUP models (GBLUP_ADD and GBLUP_ADDOM) and with standard CNN and LightGBM models for the traits egg production, egg weight, shell strength, and color. Heritability ranged from low for egg production (h^2 = 0.06–0.08) to moderate for shell strength (h^2 = 0.125–0.284) and egg weight (h^2 = 0.33–0.45). Dominance variance was generally small for all traits, but reached a maximum of 11.9% for shell strength. For traits with moderate heritability, models with the 2NP matrix achieved the highest performance. CNN2NP had the strongest predictive ability for egg weight, shell strength, and color, surpassing GBLUP by 6–18% for egg weight and 63% for shell strength, while CNN2NPSTR and CNNTrans showed smaller, inconsistent improvement compared to GBLUP. Notably, 2NPLGBM consistently ranked below CNN2NP, suggesting that convolutional architectures are more effective than tree-based boosting methods at exploiting the additive–dominance representation of the 2NP matrix. GBLUP_ADDOM performed similarly to GBLUP_ADD for most traits, indicating limited benefit from modeling dominance. For egg production traits, GBLUP remained superior, while deep learning and boosting models exhibited unstable or near-zero predictive ability. The higher performance of CNN2NP over GBLUP and other models demonstrated that performance increases arise from the interaction between the matrix transformation and the model architecture, rather than from the matrix transformation alone. Interestingly, the limited improvement observed for egg production traits highlights the reduced effectiveness of complex models for low-heritability traits. Overall, the 2NP matrix provides a way to represent additive and dominance genetic effects based on quantitative genetics principles. When combined with convolutional neural networks, it enables the model to capture complex, higher-order interactions across the genome. In this way, the approach extends classical genomic prediction methods such as GBLUP into a nonlinear method while still maintaining interpretability. Thus, the 2NP matrix offers a practical bridge between quantitative genetics and DL methods for predicting the genetic performance of complex traits.

Tailored interferon receptor knockouts in chickens reveal key regulators of disease resilience

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The global poultry industry faces increasing demands for high-efficiency production alongside the need to improve disease resilience in sustainable and socially responsible systems. However, breeding for health traits remains challenging due to their complexity, low heritability, and the difficulty of obtaining robust phenotypic data under field conditions. A deeper mechanistic understanding of immune pathways is therefore required to support next-generation breeding strategies. Recent advances in CRISPR/Cas genome editing in poultry have demonstrated the feasibility of introducing disease resistance traits, such as resistance to avian leukosis virus, highlighting the potential of targeting key immune pathways. Here, we generated genetically altered chicken models lacking receptors for type I interferons (IFNAR1) or type III interferons (IFNLR1) to investigate the role of interferon signaling in immune regulation and disease outcome. Loss of interferon signaling resulted in defined modulations in immune cell composition, including reduced circulating B cells and monocytes, and shifts in T-cell subsets. These changes were accompanied by impaired adaptive immune responses, characterized by reduced antigen-specific IgM and IgY production and altered dynamics of B- and T-cell responses following immunization. In infection experiments with avian influenza virus, type I and type III interferons exhibited non-redundant roles in host defense. IFNAR1 deficiency led to rapid disease progression, increased viral replication, dysregulated inflammatory responses, and reduced survival. In contrast, IFNLR1 deficiency showed context-dependent effects, including altered tissue-specific responses and reduced pathology in certain compartments, highlighting distinct functional specializations of both pathways. Together, these findings identify interferon signaling as a central regulator of immune system function and disease outcome in chickens. This work provides a mechanistic framework to understand variation in disease resilience, which is essential for the development of next-generation breeding strategies, although translation into practical applications remains a key challenge.

A systematic review of resilience indicator traits in chickens

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The resilience of an animal is its ability to cope with short-term disturbances, including those caused by pathogens, through response and rapid recovery to its original state. Resilience is of great relevance in – among other things – the context of a changing climate, especially in extensive production systems. To provide an up-to-date overview of the scientific knowledge on chicken resilience, a systematic literature review was conducted, focusing on 1) identifying indicator traits which have been applied in chickens to quantify or rank the birds' resilience, 2) identifying data processing methods which might be broadly applicable for studying resilience in chickens or even other species, and 3) developing hypotheses for the development of (more) practically applicable or precise resilience indicator traits in chickens. The protocol and review were conducted according to PRISMA guidelines, broken down into the common four key components: Population, Exposure, Comparator, and Outcome (PECO), to answer the question "What resilience measures have been identified in chickens?". Using predefined search criteria, including 'chickens' as the population of interest, a stress exposure description, and the term 'resilience', 760 publications were retrieved for initial screening. The screening of titles and abstracts followed a hierarchical screening process and was assisted by the AI-based tool ASReview (not replacing human verification), followed by full text screening and analysis. Following screening, 33 relevant publications on resilience indicator traits used in chickens were identified, out of 96 publications included in the full text screening. The majority of publications were small or medium scale studies and trials, and only two studies used routinely collected big data. Immune (39%) or thermal challenges (42%), especially heat stress, were most prevalent in the assessed studies. Of the studied resilience indicators, most were production- or performance-related parameters and/or immunity or disease-related parameters. Genetics- or gene expression-related indicators were reported in about half of the assessed studies, while reporting on behavioral indicators of resilience – other than feed intake – was limited (12%). Overall, the systematic review provides a comprehensive overview of published resilience indicators in chickens and highlights gaps for future research. Based on the assessed studies, we advise to additionally study stressors other than immune or temperature challenges, such as failures in water supply or handling by humans, which birds might commonly encounter in practice. This review furthermore reveals a need for improved alignment in the use of terms like resilience, robustness, resistance, tolerance or adaptability, and highlights the relevance of assessing the phase of recovery after a short-term disturbance in the context of resilience. This work was co-funded by the European Union's Horizon Europe Project 101136346 EUPAHW.

Genetic parameters for behavioural plasticity and predictability and their relationship with welfare traits in laying hens

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Animal welfare in modern poultry production is a pressing concern. The introduction of group-housed aviary systems now widely adopted in Europe has allowed new social interactions and spaces for animals to explore, yet the genetic basis of behaviour and its genetic correlation to welfare remains poorly understood. A major constraint has been the difficulty of recording individual-level behavioural phenotypes at scale. Advances in automated tracking technologies, such as Radio Frequency Identification (RFID) systems, have now made it possible to generate continuous, high-resolution behavioural profiles for large numbers of individually identified animals, opening new avenues for genetic analysis. This study takes the concepts of plasticity and predictability as novel traits in poultry, measured on a laying hen population from the HenTrack project. Derived from the longitudinal RFID-based movement records of approximately 2,250 laying hens (white and brown genetic lines), these traits capture two distinct dimensions of individual behavioural variation. Firstly, plasticity describes how an animal's behaviour changes over time as it acclimates to a new environment. Secondly, predictability quantifies how consistently an individual behaves around its own daily average. Both traits are well established in wildlife ecology and behavioural biology but have rarely been examined in a livestock genetics context, representing a meaningful conceptual and methodological novelty for the field. In previous studies, consistent inter-individual differences in plasticity and predictability were observed, and we expect a portion of this variation to be genetically based. Furthermore, predictability had a positive phenotypic correlation with keel bone fracture severity, suggesting that more predictable, less flexible animals may be less able to adapt their behaviour to avoid injury, a finding with direct implications for selection strategies aimed at improving welfare. Here, we will estimate heritability and genetic and phenotypic correlations of plasticity, predictability and keel bone fractures, a key welfare problem in laying hens. Genetic parameters will be estimated using univariate and bivariate genomic animal models (REML, ASReml), with additive genetic effects modelled via a genomic relationship matrix (G-matrix) constructed from a 60K SNP panel. To explore the underlying genetic architecture of these traits, genome-wide association studies (GWAS) will be conducted using a linear mixed-model approach on the 60K panel. Analyses will be performed separately within each genetic line, allowing us to assess whether the two lines differ in their genetic basis for behaviour. We expect these traits to show low to moderate heritability, in line with estimates previously reported for related behavioural phenotypes in this dataset and anticipate detecting genetic correlations that illuminate the relationship between behavioural traits and health outcomes. These results will provide the first genetic parameter estimates for plasticity and predictability in poultry, laying the groundwork for their potential inclusion in breeding programmes targeting welfare improvement.

Field-based phenotyping of egg laying and dual-purpose poultry lines in agroforestry systems: Implications for next-generation breeding for regenerative farm resilience

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Future poultry breeding strategies for Agroforestry and outdoor production systems should address a wide range of aspects. In addition to production efficiency important parameters such as resilience to environmental variability, climate stress, and welfare expectations are also key aspects. While genetic selection programs have historically focused on performance traits under controlled housing conditions, heritage breeds were highlighted as potential alternatives despite the lack of commercial availability. Due to the increased interest in alternative and pasture-based production systems, an evaluation was required for available poultry lines under more heterogeneous environmental contexts. This study presents a field-based phenotyping framework applied to commercial laying hens (Highlander) and dual-purpose lines (GAINA & EUREKA) managed within a rotational pastured poultry system integrated into an agroforestry setting in central Italy. The trial evaluated the adaptability and performance in mobile coops with outdoor pasture access. Each flock consisted of approximately 30 hens and one rooster, managed within electric-net enclosures across a mulberry orchard used as a multifunctional grazing environment. Data collection focused on production and resilience indicators relevant to next-generation breeding goals. Parameters included egg production (quantity and quality), feed intake, labour requirements, behavioural adaptation to pasture, and mortality events under seasonal climatic variation. Additional observations included the adaptability of each line to a short chain economic model typical to small scale agroforestry farms. Observations report limited variability in egg quantity per line with 75 – 82% of egg lying per day across all three. However, substantial variability occurred in the GAINA line, with significant variability in egg size and shell quality. Feed consumption was similar for GAINA and Highlander lines, but was 20% higher in EUREKA. The Highlander line registered higher exploratory behaviour and pasture utilization, while others showed reduced environmental engagement. Accordingly, yolk colour density (measured on 1-15 scale) and indicator for metabolic capacity for beta carotene absorption from grass was higher in Highlander (13 vs 11 in the other two). This line also required 30% more workload due to escape events. Overall mortality was minimal, with only two cases registered in GAINA line, with pathological analysis reporting egg retention and cloaca prophylaxis as causes. After completing the growth cycle, dual use lines registered above 3 kg avg weight, with EUREKA hens roughly 300g heavier, Highlander growth was economically insignificant (1.3 avg weight). However, the added value in dual purpose lines became insignificant in comparison to the increased feeding costs across the production cycle. The results underline the importance of expanding phenotyping environments beyond controlled housing conditions to support breeding strategies aligned with diversified poultry production systems. Identification of genetic traits supports the development of next-generation poultry lines suited to evolving production landscapes and regenerative farming systems.

Effects of in ovo stimulation with bioactive substances on epigenetic modifications in broiler chickens

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Early life nutritional and microbial stimulation may induce long term epigenetic effects in poultry. In ovo administration of bioactive substances represents a strategy to modulate intestinal development before hatching. Sodium butyrate (SB), a short-chain fatty acid (SCFA) naturally produced by intestinal microbiota, serves as an energy source for enterocytes and functions as an epigenetic regulator, partly through inhibition of histone deacetylases. Galactooligosaccharides (GOS), a prebiotic that selectively stimulates the growth and activity of intestinal bacteria, along with SB, are both important modulators of intestinal physiology. Due to the close interaction between the gastrointestinal tract and peripheral organs via the gut–liver axis, early exposure to this bioactive substances may influence epigenetic programming in many tissue. The liver plays a central role in metabolic regulation, immune homeostasis and remains in constant functional interaction with the gastrointestinal tract through the gut–liver axis. The aim of the study was to evaluate the effect of in ovo administration of bioactive substances on global DNA methylation levels and histone deacetylase in liver, cecal mucosa and ileum mucosa of broiler chickens. Fertilized eggs of Ross 308 broilers were injected on 12 day of incubation. Four experimental groups were established: non injected control, saline injected control (NaCl), injected by GOS and injected by SB. After hatching, birds were reared under standard production conditions until day 42. All samples were collected postmortem. Global DNA methylation was quantified in the same tissues in which histone deacetylase (HDAC) activity was determined, namely the cecal mucosa, ileal mucosa, and liver. Global DNA methylation levels were expressed as the percentage of 5-methylcytosine (5-mC) in total DNA. HDAC activity was expressed as units per gram of tissue (U/g). The obtained results demonstrated that in ovo stimulation influenced global DNA methylation in a tissue dependent manner. In the liver, a significant decrease in global methylation was observed in the GOS group compared to the NaCl group. In the SB group, methylation levels were slightly reduced compared to NaCl, although the difference was not statistically significant. In the cecum, no significant differences were detected, however, a downward trend in global methylation was noted in both the NaCl and SB groups relative to the non-injected control. In the ileum, global DNA methylation remained relatively stable in all experimental groups. No significant differences in HDAC activity were observed in the liver or ileal mucosa among the experimental groups. In contrast, HDAC activity in the cecal mucosa differed significantly between groups, with the highest value recorded in the sodium butyrate group compared to the control. These results support the hypothesis that bioactive substances applied during embryonic development may influence a long-term epigenetic effects in broiler chickens. The study was financed by grant UMO-2021/43/D/NZ9/01548 financed by the National Science Center in Cracow (Poland). Mobility financially supported by the Minister of Science under the program ”Regional Initiative of Excellence” (RID/SP/0017/2024/01).

Prenatal motor activity in layer embryos: first evidence of sex-linked differences and exploration of strain effects

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In wild birds, species differ in embryonic movement patterns. For example, brood-parasitic cuckoos exhibit substantially higher embryonic movement than their hosts, a pattern interpreted as pre-hatch "exercise" that strengthens chicks for high-force, early post-hatch tasks such as ejecting host eggs. Because embryonic movement contributes to musculoskeletal development in wild precocial species, it seems plausible that physiological priming of motor and muscle properties prior to hatch may also occur in domestic chickens. We hypothesize that genetic background including sex may influence embryonic movement in domestic strains, particularly given the distinct origins of white-feathered (White Leghorn ancestry) and brown-feathered (Rhode Island Red ancestry) commercial lines. To test this, 408 eggs (200 white-feathered strain, and 208 brown-feathered strain) were placed into a NatureForm I-series incubator (99.5°F and 55% humidity) with an automatic egg turning mechanism (rotating every 60 minutes). On embryonic day 17, eggs were removed from the incubator (1 tray at a time, 30 eggs/tray), placed onto a heated blanket (99-100°F), and covered with a 63L storage container (59.44 x 48.77 x 34.04 cm). Each egg was removed from the egg tray and individually placed into the chamber of a validated Egg Buddy digital egg monitor (Avitronics, UK); a noninvasive device whose sensors detect changes in reflected light caused by internal movement. A GoPro camera positioned 20 cm above the monitor recorded the device's display in real time. The display was recorded for 120 seconds/egg: 60 seconds for chamber habituation, and 60 seconds for movement recording. Videos were later visually scored to determine embryonic movement rate (movements/minute). A total of 330 eggs hatched into 172 females (92 brown, 80 white) and 158 males (73 brown, 85 white). Effects of sex and strain on embryonic movement rate (EMR) were analyzed using a generalized linear mixed model in SAS, including their interaction as a fixed effect. Overall EMR did not differ significantly between white and brown strains ($p=0.3178$), However, males showed higher EMR than females ($p=0.039$), driven by a strain by sex-specific effect: brown males moved more than brown females ($p=0.0316$) , whereas no sex difference was detected within the white strain, despite white males having the highest numerical EMR (107.60 ± 3.48). To our knowledge, these results provide the first documentation of sex-specific EMR in domestic, brown-feathered chickens. The absence of a main effect of strain on EMR at day 17 suggests that baseline prenatal motor activity may be relatively conserved across these two commercial white- and brown-feathered lines at this stage, despite their distinct founder ancestries. One interpretation is that strain-differences in motor phenotypes either emerge earlier or later in incubation or manifests more clearly after hatch under differing motivational and environmental contexts. Ongoing analyses at earlier incubation days and post-hatch behaviour will address these questions (and will be presented at the conference).

Temporal dynamics and sex-specific transcriptomic remodeling of chicken primordial germ cells in extended in vitro culture

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Primordial germ cells (PGCs) are vital for the introduction of genetic modification into the chicken genome and the ex situ conservation of male and female avian biodiversity. While in vitro culture is necessary for these applications, the impact of prolonged cultivation on the PGC transcriptome, particularly regarding sex-specific differences and molecular integrity, remains poorly understood. In this study, we performed single-cell RNA sequencing (10x Genomics) on gonadal PGCs derived from Lohmann LSL-classic embryos (ED 6.5). To evaluate the effects of culture, cells were analyzed at three time points: 3 days (short-term), 4 weeks (mid-term), and 7 weeks (long-term). Using a feeder-free and serum-free culture system, we analyzed 43 biological replicates (19 female, 24 male). This experimental mixture of genotypes was deconvoluted using genetic variants detection by Souporcell (v2.5). Our analysis revealed significant transcriptional shifts over time. The 4-week culture period exhibited the highest overall gene diversity, with a notably high proportion of long non-coding RNAs (~42%). In contrast, the 7-week group showed a marked reduction in total expressed genes, suggesting a 'transcriptional narrowing' during long-term maintenance. Across all time points, PGCs maintained the expression of core pluripotency-associated factors (NANOG, POU5f3, SOX3, SALL4) and canonical germline marker genes (DAZL, DND1, SMC1B, ELAVL4, DDX4). Additionally, we identified a shift in the number of differentially expressed genes (DEGs) between sexes as cultivation progressed, with the most pronounced differences appearing at 4 weeks. While PGCs remain phenotypically stable, our findings reveal significant time-dependent and sex-specific transcriptomic remodeling; identifying these functional roles and the underlying heterogeneity in in vitro cultures is a critical step toward optimizing in vitro conditions for enhanced germline transmission and the preservation of avian genetic resources via PGCs.

Quantitative genetic analysis of growth, carcass and meat traits in Sundheimer chickens

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The Sundheimer chicken is an endangered, purebred dual-purpose breed from Germany. Genetic improvement of performance traits may encourage wider use of this breed and is therefore seen to reduce its endangered status. Within the dual-purpose concept, focus is on laying traits of hens and on growth, carcass and meat traits of roosters; this ongoing study initially addresses the latter. The study aimed at estimating variance components to get a first impression on the heritable variation for these traits in the Sundheimer breed. To establish a founder population, hatching eggs were collected from 19 breeders. Using these animals, matings were subsequently carried out, based on individual performance and pedigree to avoid inbreeding. The rearing phase was carried out with mixed sexes in mobile houses until sex determination at day 64/65. Thereafter, in the years 2021–2023, the growth performance test was conducted in small groups in a fixed house until slaughter at day 119, and in 2024 in a large group in a mobile house with access to pasture until day 122. Roosters were fed organic feed throughout. Body weight (BW) was recorded at four time points: BW1 (day 24/30), BW2 (day 63–65), BW3 (day 105/107) and BW4 (day 118/121. Average daily gain (ADG) for the rearing (based on BW1 and BW2) and fattening period (based on BW2 and BW4) were derived as measures of growth performance. Carcass weight (24 h pm, CW), dressing percentage (DP), and breast (BP), leg (LP) and wing (WP) proportions were recorded. Phenotypic and genotypic data were available for 418 roosters for growth traits and 258 for carcass traits. Genotyping used the Illumina Chicken 50K BeadChip; after quality control, 37,731 SNPs remained. Raw data were Box–Cox transformed and Z-standardized prior to variance component analysis by means of mixed linear animal models. The test-year–housing-group effect was included as a fixed effect after evaluation by Wald test. The variance and covariance structure of the animal effect was modelled using a genomic relationship matrix. Descriptive analyses of growth traits yielded mean values (± SD) of BW1 293 g (±78), BW2 1,127 g (±160), BW3 2,014 g (±260) and BW4 2,296 g (±290), and ADG in the rearing and fattening phase of 23.0 g (±3.6) and 21.2 g (±4.2). Mean CW was 1,311 g (±221) and DP 58.1% (±2.4). Mean cut proportions were 17.1% (±1.2) for BP, 36.5% (±1.3) for LP and 13.7% (±1.1) for WP. All heritability estimates were significant ($p < 0.001$) and ranged from medium to high values for growth traits, with h^2 (SE) of 0.39 (0.10) for BW1, 0.56 (0.09) for BW2, 0.46 (0.09) for BW3, 0.46 (0.09) for BW4, 0.59 (0.09) for ADG during rearing and 0.41 (0.09), 0.65 (0.13) for CW and 0.41 (0.15) for DP. Heritabilities were comparably lower for BP 0.32 (0.17), LP 0.23 (0.15) and WP 0.34 (0.16). The medium to high heritabilities of the growth traits indicate a potential to breed for these traits, however, given the population makeup and limited sample size the results must be taken with caution. Larger sample sizes and a broader range of testing environments are required to draw inferences regarding genetic gain. In addition, to maintain the dual-purpose type, the genetic correlation between growth traits in roosters and laying traits in hens must be considered.

Genotype and housing systems influence Eimeria prevalence in FUNAAB Alpha and indigenous Nigerian chickens under natural infection conditions

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Coccidiosis, caused by protozoan parasites of the genus Eimeria, remains one of the most economically important diseases affecting poultry production worldwide. In Nigeria, both indigenous and improved chicken populations contribute substantially to food security, yet information on how host genetics and management systems influence Eimeria infection dynamics under natural conditions remains limited. The recently developed FUNAAB Alpha chicken was selected for improved productivity and adaptation to tropical environments, but its susceptibility to parasitic infections relative to indigenous chickens has not been evaluated. This study, therefore, investigated the prevalence and species composition of Eimeria infections in FUNAAB Alpha and Nigerian indigenous chickens and assessed the effects of genotype, sex, and housing system on infection patterns under natural exposure conditions. A total of 600 chickens were examined, comprising 300 FUNAAB Alpha and 300 indigenous Nigerian chickens representing three genotypes (normal feather, naked neck, and frizzle feather). Birds were maintained under two housing systems (deep litter and battery cage). Faecal samples were collected over a 20-week period and analysed using flotation and McMaster counting techniques to detect and quantify Eimeria oocysts. Species identification was performed using morphological measurements and shape indices under light microscopy. Oocyst count data were $\log_{10}$-transformed and analysed using generalized linear models (GENMOD procedure, SAS 9.2) to evaluate the effects of genotype, sex, housing system, and their interactions on parasite prevalence. Overall, 47.0% of sampled birds were positive for Eimeria oocysts, indicating substantial infection pressure in the study population. Indigenous chickens exhibited significantly higher prevalence (55.7%) compared with FUNAAB Alpha chickens (38.3%), suggesting population-level differences in susceptibility or exposure. Six Eimeria species were detected across populations. Eimeria tenella was the predominant species in both chicken groups, while species distribution differed between populations. Eimeria necatrix occurred more frequently in FUNAAB Alpha chickens, whereas Eimeria brunetti was detected exclusively in indigenous chickens. Prevalence of E. acervulina and E. maxima did not differ significantly between populations. Host genotype and management factors influenced infection patterns differently across populations. In indigenous chickens, oocyst prevalence did not differ significantly among genotypes, sexes, or housing systems, although slightly higher values were observed in females, frizzle-feathered birds, and deep-litter systems. In contrast, FUNAAB Alpha chickens showed significant genotype and housing effects. Birds reared under deep-litter management had substantially higher infection prevalence than cage-reared birds, and frizzle-feathered chickens showed the highest susceptibility among the three genotypes. No significant sex effects were detected. These findings demonstrate that both genetic background and management conditions shape Eimeria infection dynamics in Nigerian chicken populations.

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