



Re-Livestock
RESILIENT FARMING SYSTEMS

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An impact of including methane emission into the Polish Holstein-Friesian cattle breeding program

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Abstract

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).



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

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Abstract

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 ($22 \pm 1^\circ\text{C}$) 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



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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).



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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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Abstract

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 ($22 \pm 1^\circ\text{C}$) 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)



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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).



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Effect of ventilation regimes on carbon and nitrogen dynamics in composted bedded packs for Mediterranean dairy cattle production

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Abstract

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.



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Seasonal adaptation is associated with systemic metabolic remodelling in heavy pigs: a molecular phenomic approach

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Abstract

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



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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).



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

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Abstract

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



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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).



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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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Abstract

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



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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).



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

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Abstract

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



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RESILIENT FARMING SYSTEMS

Facilitating Innovations for Resilient Livestock Farming Systems, abbreviation: Re-Livestock (reference number: 101059609).



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

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Abstract

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



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soybean and lentil (4170, 3614, 4984 $\mu\text{mol/g} \pm 186$; $P < 0.001$). Acetate was lowest with lentil, with no differences between soybean and chickpea (2694, 3368, 3671 $\mu\text{mol/g} \pm 143$; $P < 0.001$). Butyrate was highest with chickpea, followed by lentil and soybean (409, 621, 909 $\mu\text{mol/g} \pm 105$; $P < 0.001$). Propionate did not differ among substrates (175 $\mu\text{mol/g} \pm 36$; $P > 0.10$). 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. Funded by EU project Re-Livestock (101059609).



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Effects of Temperature-Humidity Index on reproductive and productive performance in Spanish commercial sow farms

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Abstract

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



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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.



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

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Abstract

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



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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.



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Impact of pastures composition on calves microbiota in saliva, rumen and feces

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Abstract

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



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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.



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