



Re-Livestock
RESILIENT FARMING SYSTEMS

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Re-Livestock Abstracts

Multi-country genomic prediction of methane emissions in dairy cattle	4
C. I. V. Manzanilla-Pech ¹ , A. E. Van Breukelen ¹ , O. Gonzalez-Recio ² , E. Teran ² , M. Pszczola ³ , M. Ryczek ³ , T. Strabel ³ , M. L. Van Pelt ⁴ , C. Orrett ⁵ , R. F. Veerkamp ¹ , Y. De Haas ¹ , B. Gredler-Grandl ¹	
Methane emission in dairy cattle breeding programs: sensitivity of genetic parameters, economic weights and phenotyping strategies.....	5
B. Gredler-Grandl ¹ , A. E. Van Breukelen ¹ , C. I. Manzanilla Pech ¹ , Y. De Haas ¹ , R. F. Veerkamp ¹ , L. Büttgen ¹	
Innovative approach to data management of methane emissions in dairy cows	6
M. Ryczek ¹ , T. Strabel ¹ , M. Pszczola ^{1,2}	
Impact of introducing methane emission traits in the breeding goal for Polish Holstein-Friesian cattle	7
G. Cieleń ¹ , N. Pycińska ¹ , M. Pszczola ²	
Impact of including novel heat stress indicators on the genetic progress of Iberian pigs.....	8
N. Pycińska ¹ , M. Pszczola ¹ , C. Lewis ² , J. Herrero ² , E. Sell-Kubiak ¹	
Regulations and evidence requirements for authorization of enteric methane-mitigating feed additives.....	9
J. Tricarico ¹ , F. Garcia ² , A. Bannink ³ , S. S. Lee ⁴ , M. Miguel ⁴ , J. Newbold ⁵ , P. Rosenstein ⁶ , M. Van Der Saag ⁷ , D. Yáñez- Ruiz ⁸	
A guideline to uncover the mode of action of anti-methanogenic feed additives for ruminants.....	10
A. Belanche ¹ , A. Bannink ² , J. Dijkstra ² , Z. Durmic ³ , F. Garcia ⁴ , F. G. Santos ⁵ , S. Huws ⁵ , J. Jeyanathan ⁶ , P. Lund ⁷ , R. I. Mackie ⁸ , T. A. Mcallister ⁹ , D. P. Morgavi ¹⁰ , S. Muetzel ¹¹ , D. W. Pitta ¹² , D. R. Yáñez-Ruiz ¹³ , E. M. Ungerfeld ¹⁴	
Feed additives for methane mitigation: How to account for the mitigating potential of antimethanogenic feed additives - Approaches and recommendations.....	11



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Recommendations for testing enteric methane-mitigating feed additives in ruminant studies..... 12

A. N. Hristov^{1,2}, A. Bannink², M. Battelli², A. Belanche², M. C. C. Sanz², G. Fernandez-Turren², F. Garcia², A. Jonker², D. A. Kenny², V. Lind², S. J. Meale², D. Meo Zilio², C. Muñoz², D. Pacheco², N. Peiren², M. Ramin², L. Rapetti², A. Schwarm², S. Stergiadis², K. Theodoridou², E. M. Ungerfeld², S. Van Gastelen², D. R. Yáñez-Ruiz², S. M. Waters², P. Lund²

Feed additives for methane mitigation: Modeling the impact of feed additives on enteric methane emission of ruminants—Approaches and recommendations..... 13

J. Dijkstra¹, A. Bannink², G. Congio³, J. Ellis⁴, M. Eugene⁵, F. Garcia⁶, M. Niu⁷, R. Vibart⁸, D. Yanez-Ruiz⁹, E. Kebreab¹⁰

Recommendations for identification and selection of bioactive compounds to develop antimethanogenic feed additives..... 14

Z. Durmic¹, E. Duin¹, A. Bannink¹, A. Belanche¹, V. Carbone¹, D. Carro¹, M. Crüsemann¹, V. Fievez¹, F. García¹, A. Hristov¹, M. Joch¹, G. Martinez-Fernandez¹, S. Muetzell¹, E. Ungerfeld¹, M. Wang¹, D. Yáñez-Ruiz^{1,2}

Farming system is the main driver of enteric methanogenesis in grass-based veal calves 15

G. Mesbahi¹, A. K. Steiner¹, F. Leiber¹, J. Werner¹

Environmental and Economic Impacts of Extending the Productive Lifespan of Swiss Dairy Herds in Diverse Husbandry Systems 16

M. Lozano-Jaramillo¹, M. Walkenhorst¹, F. Leiber¹, C. Pfeifer¹

How nutrition can help to shape the future of Livestock Farming Systems 17

D. R. Yáñez-Ruiz¹

Challenge voice – Thinking the future food systems..... 18

W. Simon¹, H. Van Zanten¹

A comprehensive genetic evaluation of molecular phenotypes in pigs: gaining knowledge through metabolite ratios 19

S. Bovo¹, M. Bolner¹, G. Schiavo¹, G. Galimberti², F. Bertolini¹, A. Ribani¹, S. Dall'Olio¹, P. Zambonelli¹, M. Gallo³, L. Fontanesi¹

Untargeted metabolomics describes genetic factors that influence pig metabolism for adaptation traits in a Duroc line..... 20

S. Bovo¹, M. Bolner¹, C. Lewis², J. Holl³, B. Valente³, G. Schiavo¹, F. Bertolini¹, L. Fontanesi¹

Between Heat Tolerance and Resilience: Breeding Against Rising Heat Stress in Dutch Holstein Cattle..... 21





T. Pook¹, M. Ramon², C. Diaz², M. J. Carabano Luengo², M. L. Van Pelt³, L. Zetouni⁴,
C. Orrett⁴, J. Vandenplas¹, I. Adriaens⁴, C. Kamphuis¹, Y. De Haas¹, B. Gredler-Grandl¹

In vitro effects of bakery by-products on rumen degradability, fermentation, and methane yield 22

C. Christodoulou¹, H. Jalal², L. A. Crompton¹, K. E. Kliem¹, N. Davison¹, L. G. Smith^{1,3}, D. Humphries¹, I. Fusaro², S. Stergiadis¹

Comparative analysis of the cattle rumen microbiome across three countries using long-read metagenomic sequencing for methane reduction 23

D. A. L. Flossdorf¹, O. González-Recio², A. C. Bouwman¹, C. T. Ong³, E. Ross³, B. Hayes³, B. Gredler-Grandl¹

Effects of low-protein diets partially balanced in essential amino acids and pen cleaning frequency on growth, protein efficiency and carcass characteristics in growing-finishing pigs 24

A. Antonacci¹, G. Beel¹

Comparing metabolomic profiles among three Italian heavy pig breeds 25

S. Bovo¹, M. Bolner¹, G. Schiavo¹, G. Galimberti², F. Bertolini¹, A. Ribani¹, M. Gallo³, S. Dall'Olio¹, P. Zambonelli¹, L. Fontanesi¹

Effects of microalgae on in vitro rumen degradation, fermentation characteristics, and methane yield 26

C. Christodoulou¹, M. Malik¹, K. E. Kliem¹, N. Davison¹, L. G. Smith^{1,2}, S. Stergiadis¹

Assessing the sustainability and ecosystem services of farming practices of dairy cattle farms 27

A. Mantino¹, F. Biagiotti¹, M. Finocchi¹, A. Ripamonti¹, S. Righi¹, M. Moretti¹, A. Silvi¹, G. Foggi¹, M. Mele¹

Identifying shade-seeking behavioural triggers in Holstein heifers for improved heat stress management and welfare 28

X. Díaz De Otálora¹, D. A. Méndez¹, S. Sanjuan², R. Arnau², J. M. Calabuig², A. Villagrás³, S. Calvet¹, F. Estellé¹

Impact of housing design and animal level ventilation on pig growth and air quality 29

N. Hlel¹, B. Fajardo¹, C. Cano², L. Piquer², S. Calvet¹, F. Estellés¹, X. Díaz De Otálora¹

Describing adaptation to heat stress through untargeted metabolomics in pig genetic resources 30

M. Bolner¹, A. Ribani¹, S. Bovo¹, G. Schiavo¹, V. Taurisano¹, F. Bertolini¹, S. Dall'olio¹, J. Vegni¹, P. Zambonelli¹, M. Gallo², M. Pszczoła³, E. Sell-Kubiak³, L. Fontanesi¹

The metabolome, a new phenotype: a comparative study of metabolic profiles in plasma and serum 31

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Multi-country genomic prediction of methane emissions in dairy cattle

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Abstract

Genetic selection for lower methane-emitting cows is a promising strategy to reduce methane (CH₄) emissions. Achieving high accuracy in the prediction of genomic estimated breeding values (GEBV) requires a large number of animals with CH₄ records. Previous studies have shown that a reference population of at least 50,000 cows is needed to achieve a reliability of 0.6 for a trait with a heritability of 0.2. Despite numerous efforts, the number of CH₄ records within countries remains limited. However, merging datasets from different countries can enhance the accuracy of prediction of GEBV for CH₄ emissions. This study examined whether incorporating data from other countries enhances the accuracy of prediction of GEBV for CH₄ emissions. Phenotypes for CH₄ methane concentration from sniffers from the Netherlands, Spain, and Poland were merged and standardized for editing. The CH₄ dataset comprised 132,961 weekly records from 7,669 cows in the Netherlands, 12,456 records from 3,426 cows in Spain, and 7,104 records from 483 cows in Poland. Genetic correlations between countries ranged from 0.53 to 0.67. Three scenarios were proposed: (1) base scenario using only within country data (2) treating each country as a different phenotype and (3) using the collated dataset, assuming the trait is the same across countries. Validation will be done applying a forward-cross-validation approach, where the dataset is divided into two subsets—older data and newer data—with the newest subset serving as the validation population and the oldest subset as the reference population. This work has received funding from the European Union's Horizon Europe research and innovation programme, grant agreement No 01059609 (Re-Livestock project).



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Methane emission in dairy cattle breeding programs: sensitivity of genetic parameters, economic weights and phenotyping strategies

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Abstract

Reducing enteric methane (CH₄) emissions by selective breeding is key to mitigating greenhouse gas emissions from livestock farming. Designing sustainable breeding programs requires understanding the impact of genetic parameter accuracy, economic weights, and phenotyping strategies. When considering CH₄ production as the main trait and CH₄ concentration (from sniffers) as an auxiliary trait, it is more important to estimate the heritability for CH₄ production accurately, than the heritability for CH₄ concentration. When 100 progeny per bull are phenotyped with sniffers, a one s.e. lower heritability for CH₄ production than the actual estimate results in 9.8% lower genetic progress (so directed to a higher CH₄ production), whereas a one s.e. lower heritability for CH₄ concentration results only in 0.5% lower genetic progress. Sensitivity analysis showed that under ceteris paribus assumption the phenotypic variance of both CH₄ traits in the population and the genetic correlation between CH₄ production and CH₄ concentration measured by sniffers strongly influence the genetic progress for lower CH₄ emissions. A genetic correlation between CH₄ production and CH₄ concentration one s.e. lower than the actual estimated correlation results in a 23.0% higher CH₄ production. The economic value of CH₄ considered in the selection index, e.g. determined by a carbon tax applied to livestock, also strongly influences genetic progress. In the selection theory-based calculation, the height of the Danish carbon tax on dairy cattle planned for the year 2035 leads to a situation that 29.7% of the total economic response to selection results from CH₄ production. In addition, we evaluate the impact on genetic progress of different CH₄ national phenotyping strategies and the integration of CH₄ phenotypes from other countries when forming genomic across-country reference populations as tested within the Re-Livestock project (Horizon Europe, GA No. 01059609). Phenotyping strategies include testing fixed numbers of phenotyped cows with sniffers, GreenFeed and sniffers, and different genotyping proportions.



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Innovative approach to data management of methane emissions in dairy COWS

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Abstract

Methane (CH₄) is a potent greenhouse gas linked to climate change, with ruminant livestock being a significant source of emissions. Accurate phenotyping of methane emissions in dairy cows is essential for developing mitigation strategies, such as selective breeding for lower emissions. In this study, we developed a data processing pipeline for methane measurements collected using sniffer devices installed in automated milking systems. The dataset included methane (CH₄) and carbon dioxide (CO₂) concentration measurements recorded every 7 seconds during milking sessions in Polish Holstein-Friesian cows at two commercial farms in western Poland. Data were collected over approximately 20 months on Farm 1 and around 6 weeks on Farm 2. The proposed pipeline consists of two key steps: (1) precise identification of milking start and end times using peak detection methods and (2) background correction based on stable methane plateau regions to account for ambient CH₄ variability. Visual inspection confirmed improved accuracy in detecting milking start and end times compared to previous methods, as well as a more reliable identification of non-milking periods. This approach enhances the precision of methane emission phenotyping in dairy cows. This work was funded by the European Union's Horizon Europe research and innovation program under grant agreement No. 1059609 (Re-Livestock project).



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Impact of introducing methane emission traits in the breeding goal for Polish Holstein-Friesian cattle

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Abstract

Livestock, especially ruminants, produce methane and other greenhouse gases, contributing to climate changes. One potential solution to reduce methane production is selective breeding. Yet, the impact of including methane traits into breeding goals is largely unknown. In Polish Holstein-Friesian cows the selection index consists of six sub-indices: production traits and somatic cell count, conformation traits, fertility traits, calving ease-calf survival, longevity, milk ability traits. The aim of this study was to evaluate the effect of incorporating methane emission traits into selection index for Polish Holstein-Friesian cattle on the genetic gain of traits currently included in the breeding goal and the potential of reducing greenhouse gas production in the next generation. The simulation of various breeding scenarios was done using the Modular Breeding Program Simulation (MoBPS) package in R. The following strategies were used: selection based on Polish-Holstein breeding program and compare to additional selection that included methane emission. Genetic parameters were obtained from existing breeding program. Genetic correlation between methane emission and these traits were also taken into account, and various scenarios for the impact of selection intensity and the weighting of traits were evaluated. The finding from this study, can be used to evaluate the effect of inclusions methane emission into cattle breeding program. 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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Impact of including novel heat stress indicators on the genetic progress of Iberian pigs

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Abstract

Climate change has increased heat stress (HS) events affecting pig production. Knowledge on impact of including HS resistance traits in current breeding programs is limited. We aimed to examine the impact of including novel HS resistance trait, based on biomarkers, into the breeding scheme of Iberian pigs. Various scenarios were simulated using the MoBPS package in R. Scenarios considered distinct traits for males and females. For males, average daily gain and loin depth were included. Females' traits included litter size, stillborn, wean-to-estrus interval, number of teats, pre-wean mortality, and individual piglet birth weight. Back fat was included for both sexes. Breeding program was based on a maternal selection scheme, focusing on reproductive traits, while other traits were monitored as correlated changes but excluded from selection. Heritabilities, genetic variances, and phenotypic means were obtained from studies or existing Iberian pig breeding programs mimicking the current breeding scheme. Scenarios varied in terms of reference population size (500, 1000, 2000, 3000, 4000, 5000), selection criteria (phenotypic vs. EBV), selection intensity (1:10, 1:20, 5:10), and economic weights assigned to reproductive traits, with and without HS resistance traits. The impact of including these traits on genetic progress was analyzed to determine the best breeding strategy and feasibility of including HS traits in breeding programs for local pig breeds. This work has received funding from the European Union's Horizon Europe research and innovation programme under the grant agreement No 01059609 (Re-Livestock project).



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Regulations and evidence requirements for authorization of enteric methane-mitigating feed additives

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Abstract

A review of the regulatory and evidence requirements for authorizing anti-methanogenic feed additives (AMFA) for ruminants was conducted for seven jurisdictions. The objectives were to provide an overview to help applicants and scientists understand evidence requirements to meet regulations, identify differences and similarities between jurisdictions, and provide recommendations. Regulations protect animal health, promote food safety, and prevent unsafe practices and misleading claims. These mandatory rules cover ingredient safety, manufacturing practices, product labeling, and permissible limits for substances. The intended use is the specific purpose for which AMFA are authorized and determines the evaluation and approval process. This includes practical aspects of administration like active ingredients, dose, mixing, and feeding frequency. Each jurisdiction has unique criteria for legally classifying AMFA, making it challenging to meet all classifications with the same scientific evidence. However, all jurisdictions need robust evidence on efficacy, safety, and product quality, despite different study requirements. The applicant must provide the scientific evidence. Applicants and scientists must consult with authorities before designing studies. Scientists conduct efficacy and risk assessments following good scientific practices that inform regulatory decisions. Collaboration with regulatory agencies may refine legal classifications to improve harmonization. Educating stakeholders about AMFA's advantages and proper use encourage correct usage increasing understanding and transparency.



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A guideline to uncover the mode of action of anti-methanogenic feed additives for ruminants

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Abstract

We discuss guidelines of research required for understanding the mode of action of anti-methanogenic feed additives (AMFA). According to their mode of action, AMFA can be classified into 4 categories: 1) lowering H₂ production; 2) inhibiting methanogens; 3) promoting alternative H₂-incorporating pathways; and 4) oxidizing methane. In-depth research should be conducted only after the AMFA effectiveness and safety have been established. Research questions guiding this investigation should cover 5 perspectives: 1) microbiology, identifying the targeted microbes and the potential side effects on non-targeted microbes; 2) cell and molecular biochemistry; identifying the active compounds, elucidate mechanisms of action on targeted molecules, and investigating microbial resistance and adaptation; 3) microbial ecology, analyzing the effects of on microbial communities, shift in metabolic pathways, accumulation of fermentation products and AMFA degradation; 4) animal metabolism; studying effects on DMI, digestibility, absorption, metabolism, excretion, and safety; and 5) holistic approach; describing the modulatory effects of the diet, type of animal and management on the mode of action and effectiveness. In conclusion, complementary approaches are required to fully understand the mode of action of AMFA at various levels, moreover addressing critical knowledge gaps is essential for the successful adoption of AMFA.



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Feed additives for methane mitigation: How to account for the mitigating potential of antimethanogenic feed additives - Approaches and recommendations

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Abstract

Here we examine the approaches used for accounting of enteric CH₄ abatement through the use of AMFA in livestock systems across scales. This accounting process starts with the ruminant animal, with estimates obtained from simple empirical emission factors or equations, to complex process-based models. The accounting needs to consider the potential synergies and trade-offs in GHG emissions at levels before and after being offered to the animal. The choice of methodology and level of complexity must be tailored to the targeted scale of analysis, the availability of input data and the accounting objectives. Assumptions on the additivity of AMFA abatement should follow insights from experimental work, and in the future, from models as these continue to improve the simulation of combined AMFA efficacy and effectiveness. Collectively, the accounting of enteric CH₄ abatement by feed additives remains to be fully assessed beyond experimental results to address pragmatism, potential for adoption and societal acceptance.



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Recommendations for testing enteric methane-mitigating feed additives in ruminant studies

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Abstract

There is a need for rigorous and scientifically-based testing standards for enteric methane mitigation technologies, including anti-methanogenic feed additives (AMFA). Recommendations include study design and statistical analysis of the data, interactions with diet composition, associative effect of AMFA with other mitigation strategies, appropriate methods for measuring methane emissions, production and physiological responses to AMFA, and effects on animal health and product quality. Animal experiments should be planned based on clear hypotheses and experimental designs must be chosen to best answer scientific questions, with pre-experimental power analysis and robust post-experimental statistical analyses being important requisites. Experimental conditions should be representative of the production system of interest, to ensure that results and conclusions are applicable and practical. To explore additivity and synergism, as well as trade-offs, including relevant manure emissions, AMFA may be combined with other methane mitigation strategies and studied in appropriately designed experiments. Methane emissions can be successfully measured, and efficacy of AMFA determined, using respiration chambers, the sulfur hexafluoride method, and the GreenFeed system. For proper assessment of an AMFA, it is critically important that representative animal production data are collected and reported. In addition, evaluating the effects of AMFA on nutrient digestibility, animal physiology, animal health and reproduction, product quality, and how AMFA interact with nutrient composition of the diet is necessary and should be conducted at various stages of the evaluation process. The authors emphasize that enteric methane mitigation claims should not be made until efficacy of AMFA is confirmed in animal studies designed and conducted considering the guidelines provided.



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Re-Livestock
RESILIENT FARMING SYSTEMS

Feed additives for methane mitigation: Modeling the impact of feed additives on enteric methane emission of ruminants—Approaches and recommendations

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Abstract

Antimethanogenic feed additives (AMFA) can considerably mitigate enteric methane (CH₄) emissions from ruminants. Mathematical models are essential for understanding and predicting the quantitative impact of AMFA across diverse diets and production systems. This study provides a comprehensive overview of modelling methodologies, concluding with recommendations for quantifying AMFA's impact in support of farm-level, national, regional, and global inventories. Key considerations encompass the type of models employed (i.e., empirical, machine learning, and mechanistic models), alignment of modeling objectives, data availability and quality, modeling synergies and trade-offs associated with using AMFA, and model applications for enhanced understanding, prediction, and integration into higher levels of aggregation. Careful evaluation of additive dosage, delivery methods, and transient effects is essential, the chosen modeling approach should align with specified objectives, and synergies of diverse modelling methodologies should be used to improve understanding and accurately predict AMFA's impact on CH₄ emissions.



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Recommendations for identification and selection of bioactive compounds to develop antimethanogenic feed additives

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Abstract

This work provides technical recommendations and guidelines for research to identify and screen different compounds to reduce enteric methane (CH₄) emissions before they can be further assessed in vivo. For the initial identification of anti-methanogenic feed additives (AMFA) candidates, two approaches can be used: mechanistic (combining computational tools -in silico- and docking studies, using a modelling technique to predict how target enzymes interact with candidate molecules, and then synthesizing the candidate compounds), and empirical (finding published evidence of specific bioactive effects, by searching different repositories, followed by obtaining bioactive material from the identified source). While both approaches offer potential to identify a large number of compounds, sophisticated equipment and specific expertise are required for computational/modelling work and extracting bioactive compounds from a biological matrix, respectively, in mechanistic and empirical studies. Once candidates have been identified, the next step is to evaluate experimentally the activity and there is an array of in vitro methodologies that can be used for this purpose (enzymatic assays, pure cultures of methanogens batch cultures and continuous or semi-continuous cultures). We will present and discuss the main aspects to consider when using different approaches, including identifying the target enzymes to study, developing the enzyme material for running inhibition kinetics, culturing conditions and selection of archaeal species to study and the processing of the rumen inoculum used for experiments conducted using batch cultures and fermenters. Critical overarching methodological considerations for all in vitro approaches are: i) appropriate experimental design and statistical tests, ii) selection of doses depending on the nature and mode of action of the AMFA, iii) careful preparation of solid or liquid formats for appropriate delivery of the additives and iv) when and how to measure CH₄ and units to express its production.



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Farming system is the main driver of enteric methanogenesis in grass-based veal calves

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Abstract

Developing grass-based veal production from male dairy calves could be a low-input solution for raising such animals, particularly in organic systems. However, the impact of grazing on enteric methane emissions of young animals is not well assessed, yet. The study included three genotypes – Brown Swiss, Limousin × Brown Swiss, and Swiss Fleckvieh – raised in four distinct farming systems: a hay-based indoor system and three pasture-based systems, representing intensive, permanent, and alpine grasslands. While all grazing calves received comparable amounts of maize and alfalfa pellets, and a limited soy-free concentrate, the indoor control group followed a slightly different feeding regimen. Hay was provided ad libitum in all systems. Each genotype × system interaction comprised four male calves, raised together from day of life 90 to 180 in the respective systems. Rumen fluid was sampled by intubation after 11 weeks. In vitro gas production (ml/200 mg DM), absolute methane emissions (mg), and methane yield (mg/ml) were analysed after 24h of incubation at 38°C in a batch system. The time between sampling of the rumen fluid and start of the test run in vitro was different due to physical distances between sites and laboratory. It was included as a correction variable in the model. Analysis revealed a significant impact of the farming system on methanogenesis, whereas genotype effects were marginal and never significant. Total gas production volume was highest in the intensive grassland system and lowest in the alpine system (46.2 ml and 44.6 ml, respectively). Methane production was lowest in the indoor and intensive grassland systems, whereas the permanent and alpine grassland systems produced the highest levels—showing increases of 16% and 8%, respectively, in methane production (mg) compared to the indoor system. These results show relative differences between systems and genotypes. Scaling to emission values per unit of product is not possible, though. Further research on dietary strategies, economic feasibility, and land use efficiency is necessary to assess the sustainability of grass-based veal production.



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RESILIENT FARMING SYSTEMS

Environmental and Economic Impacts of Extending the Productive Lifespan of Swiss Dairy Herds in Diverse Husbandry Systems

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Abstract

Ruminants are major contributors to greenhouse gas emissions due to methane production and biodiversity loss through land for feed and fodder production. Prolonging the productive lifespan of dairy cattle is a promising approach, as it reduces the need for replacement animals, and the related environmental impact during the first two unproductive years. Yet, herd-level productive lifespan affects both milk productivity and economic impacts on farms. Impacts of increasing dairy cow productive lifespan on greenhouse gas emission, land use and the gross margin contribution are quantified with different representative Swiss farms. These represent different agro-ecological zones with different dairy breeds in both conventional and organic productions systems. To derive herd level yearly average milk yield from herds with short, average and long lifespans for each representative farm Swiss herdbook data was used. Greenhouse gas emissions, land use, and farm profitability were assessed using the FiBL FarmLCA tool. Prolonged productive lifespan reduces greenhouse gas emissions per unit of milk by decreasing the need for replacement animals, even when the prolonged productive lifespan comes with a reduced herd level milk yield. Land-use per dairy production unit is always reduced as well. Economically, short lifespan of dairy cows always yields lowest while average lifespan yields for most farms the highest gross margin per cow. In case of long lifespan, income lost from fewer culled cows is not offset by additional revenue. This perspective, however, ignores that the spare agricultural land could be used differently and compensates for economic loss further. In intensive systems like Holstein herds in the lowlands long lifespan yields the highest gross margin. Extending dairy cow lifespan offers clear environmental benefits by lowering emissions and land use, supporting climate and biodiversity goals. Economic viability depends on effective land use strategies and making prolonged lifespan a profitable solution for reducing CO₂ emissions in the livestock sector.



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How nutrition can help to shape the future of Livestock Farming Systems

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Abstract

The extensive research in nutrition in European livestock farming systems has greatly contributed to enhance their sustainability; however, the new societal demands, the effects of climate change and the unstable geopolitical context require co-developing new vision and approaches with relevant stakeholders. While feeding (feeds production and digestion) has a major contribution to the environmental impact of livestock systems, opportunities arise to develop science-based solutions to align with the new pathways of progress to sustainability: efficiency, circularity and diversity. This work will present the main efforts developed by different European projects (Re-Livestock, Step-Up, Pathways, HoloRuminant) and other international initiatives towards those pathways. Efficiency: Optimization of the livestock production systems through improvement of the feed conversion ratio is critical but needs to be extended to the concept of environmental efficiency. This requires a stronger collaboration across agronomy in feed production, animal nutrition and environmental assessment to fully capture the improvements. The collaboration with the Global Feed LCA Institute is a good example of joined efforts. Feed additives to reduce enteric methane emissions is a reality nowadays but still faces important challenges to extend their use: effective use of excess rumen hydrogen when methanogenesis is inhibited, accurate accounting in inventories for the reduction in emissions, full understanding of the registration and regulation frameworks and careful consideration of the communication strategies to avoid misinterpretation. Circularity: the ability of livestock animals to make use of biomass (i.e. food industry by-products as feed resources) has been largely demonstrated. However, the food processing industry is constantly evolving and generates new materials that need to be evaluated. Further efforts are being made to accurately quantify the reductions in GHG emissions associated to the use of by-products. Diversity needs to be considered at different levels. A critical aspect is to account for the future climate scenarios in different geographic regions and systems to deploy strategic vision of future feeding resources and

practices that specifically fit into those climate projections (i.e. grasslands management).



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Re-Livestock
RESILIENT FARMING SYSTEMS

Challenge voice – Thinking the future food systems

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Abstract

Feeding a growing global population within planetary boundaries requires transformative approaches to redesigning food systems. Two key strategies — circularity (optimizing natural resource use) and the protein transition (reducing animal-based food) — are widely proposed for their environmental benefits. Using a food system modeling approach, we found that minimizing environmental impacts significantly reduces livestock numbers. However, livestock remains essential at lower levels for upcycling low-cost biomass (e.g., grass, by-products, and food waste) and ensuring nutrient-adequate diets. Regional differences are substantial, making location-based solutions critical for achieving the optimal balance between animal and plant-based foods. Adopting these circularity and protein transition strategies can greatly reduce agricultural land use and greenhouse gas emissions. Envisioning future livestock farming systems necessitates a holistic approach to ensure planetary health and adequate diets at a food system level.



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A comprehensive genetic evaluation of molecular phenotypes in pigs: gaining knowledge through metabolite ratios

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Abstract

Metabolomics has emerged as a novel high-throughput approach for measuring all molecules at the basis of metabolism, providing an unprecedented picture of molecular phenotypes that contribute to describe the molecular phenome of an organism and define genetically determined metabolites. Within the molecular domain, molecules do not act in isolation but rather as part of networks. Specifically, two metabolites can be interconnected as substrates and products of an enzymatic reaction. Therefore, by exploiting this link, we can create new phenotypes (i.e. metabolite ratios) that expand the molecular phenome and provide novel insights into the molecular phenotype landscape. In this study, we obtained the metabolomic and genomic profile of ~700 Italian Large White pigs. Metabolite levels of 722 plasma metabolites were obtained using an untargeted metabolomic platform from Metabolon and genotypes were obtained with the Illumina 60k panel. Metabolite genome-wide association studies (mGWAS) were performed using the GEMMA tool. We then applied a powerful strategy that modelled metabolite relationships in the form of metabolite ratios, resulting in over more than 250,000 new molecular traits that underwent GWAS. The analysis of single metabolites identified several metabolite QTL (mQTL) regions involving 236 molecules and ~80 genes already known to be functionally linked to those metabolites, while metabolite ratios uncovered ~10 additional mQTL for additional 120 metabolites (and 450 ratios). Overall, by applying this powerful strategy, we expanded our knowledge of the molecular phenome, and its genetic control by ~300 times and uncovered new markers that can serve as proxies for more complex phenotypes. Acknowledgments: This study has received funding from the European Union's Horizon Europe research and innovation programme under grant agreement No. 01059609 (Re-Livestock project).



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Untargeted metabolomics describes genetic factors that influence pig metabolism for adaptation traits in a Duroc line

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Abstract

To enhance selection programs and develop novel breeding strategies, the pig production sector continuously explores new phenotypic traits, expanding the traditional phenotypic landscape, which has typically focused on final phenotypes such as production and performance traits. Final phenotypes are complex, influenced by many external and molecular interactions at the organismal level. These influences can be broken down by studying molecular layers or intermediate phenotypes, such as the metabolome. This study aims to genetically dissect pig metabolism to identify markers associated with the adaptation to heat-stress conditions. About 700 blood plasma metabolites were quantified through an untargeted metabolomic platform on 300 growing male Duroc pigs. Additionally, genotypes for ~40,000 single nucleotide polymorphisms were obtained for the same animals. The pigs were from two groups with extreme and divergent values for feed intake under heat-stress conditions. Genomic heritability estimates and genome wide associations for the level of metabolites were calculated using GEMMA. Heritability estimates ranged from 0.0 to 0.83 (average = 0.18; s.d. = 0.15). We found suggestive ($P < 5.5 \times 10^{-5}$) and significant ($P < 1.3 \times 10^{-6}$) marker associations for 400 and 102 metabolites, respectively. Most of these associations were between markers in enzyme-encoding genes and metabolites that either serve as substrates or final products of the corresponding genes. Overall, these newly identified genetic determinants of pig metabolism serve as (i) a novel source of information for indirectly explaining complex traits, including resilience to heat stress, proxied using feed intake measures; and (ii) new genetic markers for fine-tuning selection programs to enhance the sustainability and resilience of pig production. Acknowledgments: This study received funding from the European Union's Horizon Europe research and innovation program under grant agreement No. 01059609 (Re-Livestock project).



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Re-Livestock
RESILIENT FARMING SYSTEMS

Between Heat Tolerance and Resilience: Breeding Against Rising Heat Stress in Dutch Holstein Cattle

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Abstract

Heat stress is a major environmental challenge in dairy cattle, leading to behavioral changes, production losses, and welfare concerns. While short-term management strategies can mitigate heat stress, genetic selection can aid these efforts long term. However, breeding against heat stress is complex, as it requires balancing heat tolerance—the ability to maintain production in hot conditions—and heat resilience—the ability to reduce production to avoid severe consequences such as morbidity and mortality. Within the projects Re-Livestock (Horizon Europe, GA No. 01059609) and Breed4Food (TKI Agri & Food, LWV20054), data from 1,478 farms including 677,318 cows from 2013 to 2021 across the Netherlands were used to quantify heat stress effects based on the temperature-humidity index (THI). At THI = 75, milk yield, fat concentration, and protein concentration each decreased by 5% compared to thermoneutral conditions, with heritabilities for heat tolerance traits between 0.05 and 0.12. Improving the population through breeding by one genetic standard deviation was estimated to reduce the effect of heat stress by 11% for milk yield, resulting in a remaining relative loss of 4.5% at THI = 75. Even though the same genetic improvement offset 65% of the losses in fat and protein concentration, greater heat tolerance for these traits was negatively correlated with health, resilience, and production. In contrast, heat tolerance based on milk yield showed positive correlations to health, resilience, and longevity with no significant correlations to production. Although heat tolerance traits are highly polygenic, QTLs on BTA5, BTA14, BTA20 were identified through a GWAS using SNPs and haplotype blocks. As milk yield losses increased quadratically with THI and breeding could only offset a small share of production losses, introgression or crossbreeding with more adapted breeds may be required when breeding for more challenging climate conditions.



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Re-Livestock
RESILIENT FARMING SYSTEMS

In vitro effects of bakery by-products on rumen degradability, fermentation, and methane yield

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Abstract

Feeding bakery by-products (BBPs) to ruminants supports circularity and waste management. This study examined in vitro effects of BBPs on rumen degradation, fermentation parameters, and gas and methane output. A control (CON) diet included dried grass silage:wheat grain:soybean meal:corn distillers' grains at 600:300:70:30 g/kg dry matter (DM). Wholemeal crumbed bread (WMB), white crumbed bread (WB), cracker feed (CF), flavoured cracker feed (FCF), and biscuit meal (BM) were added at 205-215 g/kg DM, partially replacing wheat and in WMB, WB, FCF, and BM, also soybean meal. BBP diets had less starch and more fat. CF, FCF, and BM had more water-soluble carbohydrates. Three runs were conducted, each with 1g of diet incubated in Wheaton flasks with rumen fluid (four donor animals; one animal/flask):fermentation medium (10:90) for 72h, as required to model fermentation kinetics. Gas pressure (psi) was recorded and gas samples taken for methane analysis at 2, 4, 6, 8, 10, 12, 24, 32, 48, and 72h. After 72h, flask content was filtered, pH recorded, in vitro DM degradability (IVDMD) measured, and filtered content analysed for volatile fatty acids and ammonia nitrogen. The linear mixed model had treatment as fixed, and animal ID and run as random factors. Fisher's Least Significant Difference test was used for pairwise comparisons. Gas and methane curves were modelled to assess fermentation kinetics. IVDMD was higher ($P=0.007$) in WB than in WMB, FCF, and BM; and in CON than in WMB. Propionate concentration was higher ($P<0.001$) in WMB, WB, CF, and BM than in CON. Valerate concentration was higher ($P=0.004$) in WMB and WB. Acetate:propionate was higher ($P<0.001$) in CON than in WMB, WB, CF, and BM. Methane yield (mL/g IVDMD) was lower ($P=0.045$) in WB and FCF than in CON by 10%. BBP-containing diets maintained effective rumen fermentation compared to the wheat and soybean they replaced, while WB and FCF also showed methane mitigation potential.



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Comparative analysis of the cattle rumen microbiome across three countries using long-read metagenomic sequencing for methane reduction

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Abstract

The rumen microbiome plays a role in ruminant health and methane emissions, yet country-specific differences in the rumen microbiome remain largely unexplored using modern sequencing techniques. This study used long-read metagenomic sequencing to analyze the rumen microbiome of dairy cattle in The Netherlands (NL, n=961 Holstein) and Spain (ES, n=445 Holstein) and the oral microbiome of beef cattle from Australia (AU n=218 Brahman/Brahman-cross) and compared them. Methane emission (measured via Sniffer in NL/ES or GreenFeed in AU) and genotype data were integrated to explore potential methane mitigation strategies useful across countries. We assessed microbial diversity across locations, to quantify intra- and inter-country variation and explored the association between microbial taxa or gene abundances, with methane output. Genotype data further enabled estimates of heritability of microbial abundances and cross-country genetic correlations. Preliminary results indicate country-specific microbiome differences, suggesting that environmental and farm management factors may shape microbial communities. Moreover, we identified microbial taxa and genes correlating with methane emissions and heritability estimates suggest a genetic influence on microbial composition. This study provides novel insights into geographical and genetic drivers of the rumen microbiome and its impact on methane emissions, supporting breeding and management strategies to reduce cattle's environmental impact. This work received funding from: the EU's Horizon Europe research and innovation program under the grant agreement No 01059609 (Re-Livestock), the Knowledge and Innovation Agenda of the Ministry of Agriculture, Fisheries, Food Security and Nature, the COST Action CA22112- European Network on Livestock Phenomics and the Meat and Livestock Australia MDC project P.PSH.2010.



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RESILIENT FARMING SYSTEMS

Effects of low-protein diets partially balanced in essential amino acids and pen cleaning frequency on growth, protein efficiency and carcass characteristics in growing-finishing pigs

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Abstract

Reducing dietary crude protein (CP) in pig production can improve sustainability by increasing nitrogen (N) utilization, but low-protein (LP) diets must be fully balanced in essential amino acids and fed under stress-free conditions to maintain pig growth and carcass quality, otherwise performance may suffer. Therefore, forty-eight Swiss Large White female pigs (20 ± 2.2 kg BW) were reared in pens cleaned daily (D) or twice a week (W) and fed standard (ST) or LP diets according to a 2×2 factorial design. The ST grower and finisher diets were formulated to be isocaloric and contain 16.4 and 12.5% CP, respectively. The LP diets were obtained by reducing CP and digestible Lys, Met, Thr, Trp, and Val levels by about 20% compared to the respective ST diets. Weekly BW, feed intake (FI), and carcass composition by dual-energy x-ray absorptiometry were assessed for each pig. Data were analysed by linear mixed models (R, v.4.4.1), with diet, pen cleaning protocol and the interaction as fixed effects, and the litter of origin as random effect. The pen cleaning protocol influenced average daily feed intake and average daily gain. Compared to D, W pigs ate 8.4% more feed and grew 8.7% faster ($p < 0.01$), but total FI was similar as D pigs spent 5.9 more days on feed ($p < 0.01$), resulting in comparable feed efficiency. Regardless of the pen cleaning status, LP pigs were 7.5% less feed efficient, consuming 9.1% more feed and growing 4.9% less per day ($p \leq 0.03$). Carcass protein deposition efficiency was 5.4% lower in W than D pigs and 10.4% higher in LP than ST pigs ($p \leq 0.02$). Carcass fat content was 6.3 and 10.7% higher in W and LP pigs compared to D and ST pigs, respectively ($p \leq 0.04$). In conclusion, partially balanced LP diets improved protein efficiency but impaired growth and carcass characteristics. Despite growing faster, W pigs had fatter carcasses and lower protein deposition efficiency which tended to be more evident when pigs were fed partially balanced LP diets.



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Comparing metabolomic profiles among three Italian heavy pig breeds

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Abstract

The phenotypic characteristics of an animal are the result of complex molecular interactions between different layers of biological complexity, including the genetic background and intermediate phenotypes. These intermediate phenotypes describe the molecular phenotype, which includes the transcriptomic, proteomic and metabolomic profiles. The final objective of this study was to identify novel breed-specific markers by exploring the pig plasma metabolome. We applied untargeted metabolomics to characterize the plasma metabolomic profile of 168 animals from three Italian heavy pig breeds: Italian Large White (ILW; n. 56), Italian Duroc (IDU; n. 56) and Italian Landrace (ILA; n. 56). These animals were sib-tested pigs, included in the selection programs of Italian heavy pigs run by the Italian Pig Breeders Association (ANAS). The pigs were raised in the same performance station and slaughtered at nine months of age (approx. 160 kg live weight). The metabolomic profiles, which included ~700 metabolites, underwent quality checks and were analyzed using a machine learning approach (Random Forest) to identify metabolites that could discriminate between pairs of breeds. In total, we identified 84, 19 and 10 metabolites for the ILW-IDU, ILW-ILA and IDU-ILA comparisons, respectively. These metabolites clearly separated the pig breeds when tested in an unsupervised manner, reflecting the different genetic backgrounds of the three breeds. Acknowledgments: This study has received funding from the European Union's Horizon Europe research and innovation programme under grant agreement No. 01059609 (Re-Livestock project).



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Effects of microalgae on in vitro rumen degradation, fermentation characteristics, and methane yield

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Abstract

Microalgae are rich in bioactive compounds and are therefore evaluated for their potential to enhance animal health and productivity while also serving as methane mitigators. This study investigated the effects of four microalgae, namely *Dunaliella salina* (DUNA), *Spirulina platensis* (SP), *Nannochloropsis gaditana* (NANNO), and *Odontella aurita* (ODO) on rumen degradation, fermentation characteristics, and methane yield compared to a diet without microalgae (CON) using a 72-hour in vitro gas production system and three runs. The CON diet consisted of dried, ground grass silage, wheat grain, soyabean meal, and brewers' spent grains. Microalgae partially replaced soyabean meal, except in SP and the combination of DUNA+SP (DUSP), where soyabean meal was completely replaced. Wheat was partially replaced in all treatments except DUSP. Diets were isonitrogenous. Rumen fluid samples collected from four donor animals were strained and incubated with a fermentation medium at a 10:90 ratio (n=4 replicates/treatment). A linear mixed model was used, with treatment as a fixed factor and cow ID and run as random factors. Fisher's Least Significant Difference test was used for pairwise comparisons. In vitro dry matter degradability (IVDMD), ammonia concentration, and pH did not differ ($P>0.05$). Acetate, propionate, and valerate concentrations (mM) were reduced ($P<0.05$) in ODO and DUSP compared with CON, while propionate concentration was also lower ($P=0.014$) in NANNO than in CON. The propionate-to-acetate ratio and methane yield (g/kg substrate and g/kg IVDMD) did not differ ($P>0.05$). These findings suggest that microalgae inclusion does not significantly alter rumen degradation or methane production under in vitro conditions. The reduction in specific volatile fatty acids in ODO and DUSP needs further investigation to determine implications for rumen metabolism. In vivo trials should assess the long-term effects on efficiency, animal performance, and methane emissions under practical feeding conditions.



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Re-Livestock
RESILIENT FARMING SYSTEMS

Assessing the sustainability and ecosystem services of farming practices of dairy cattle farms

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Abstract

As part of the Italian Living Lab activities within the HE Project RE-LIVESTOCK (GA N° 101059609), this study examined the sustainability of agricultural practices adopted by selected dairy farms. The multicriteria analysis focused on key environmental, economic, and social aspects to identify potential trade-offs between sustainability dimensions. To achieve this, the RE-LIVESTOCK Tool, an online software based on the existing PG-Tool, was employed. Through a multicriteria survey, this flexible tool provided a comprehensive assessment of farm and land management practices, offering valuable insights for farmers, technicians, researchers, and land managers. The tool generated a sustainability score ranging from 1 to 5, providing a structured evaluation of different farming dimensions using 13 indicators covering areas such as soil and water management, animal welfare, landscape features, and social capital. The study assessed ten semi-intensive dairy farms located in the Tuscany region, Italy. Farm sizes ranged from 40 to 1600 hectares, with livestock units per farm varying from 24 to 500. The most common breed was the Italian Holstein. The results showed an average sustainability score of 2.84, ranging from 2.46 to 3.41 across all farms. Strengths were observed in soil management and animal welfare (with average scores of 3.3 and 3.85, respectively), while weaknesses emerged in nutrient balance (NPK budget, average score 1.90) and cropping system diversity (average score 2.35). Multivariate statistical analyses indicated clear distinctions in farm practices, particularly in soil management and economic performance, while also emphasizing the need for improved collaboration and social capital among farmers. In conclusion, while notable sustainability practices were observed, particularly in animal health and farming business resilience, significant improvements are needed in nutrient management and social collaboration to further enhance the overall sustainability. The RE-LIVESTOCK Tool has proven to be a valuable resource for identifying key areas and offering a pathway for farms to adopt more sustainable practices.



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Re-Livestock
RESILIENT FARMING SYSTEMS

Identifying shade-seeking behavioural triggers in Holstein heifers for improved heat stress management and welfare

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Abstract

New technologies, such as precision livestock farming (PLF) and machine learning (ML), are transforming the livestock sector by enhancing efficiency, improving animal welfare, and enabling data-driven decision-making for better farm management. In the current context of climate change, identifying behavioural triggers in response to heat stress events is an essential prerequisite for improving animal welfare and optimising farm management strategies. Therefore, by including behavioural triggers in predictive models, the development of context-specific heat mitigation strategies, cooling interventions, and farm management decisions could be facilitated. To this end, this study investigates the environmental conditions that trigger shade-seeking behaviour in Holstein heifers, using PLF technologies and ML algorithms. In contrast to the THI value of 72, our results reveal that for this specific context, a THI of 76 acts as a behavioural trigger for shade-seeking behaviour. In addition, it seems that the heat load during the last 4 days, also defines animal behaviour at a given moment. Our findings also reveal an adaptation window, where behavioural responses stabilise after extended heat exposure, potentially indicating acclimatisation of the animals to heat. Moreover, our results underscore the importance of cumulative THI values rather than relying solely on momentary observations. In contrast to one-fits-all solutions, understanding the nuanced and context-specific responses of animals is key for their welfare, resilience, and productivity in environments where heat stress compromises livestock production.



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Impact of housing design and animal level ventilation on pig growth and air quality

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Abstract

Housing design and ventilation management in pig fattening systems may affect production performance and air quality, mainly under heat stress conditions. This study evaluates the impact of floor type and auxiliary ventilation on pig growth and air quality. The experiment was conducted at the experimental farm CITA-IVIA (Segorbe, Spain) with 128 Duroc x (Landrace x Large White) pigs, divided into a control group (C) with slatted floor and an experimental group (EXP) with mixed floor (33% solid) and additional ventilation at the animal level in the last feeding phase. Animal growth, performance and emissions of NH₃ and CO₂ at the room level were measured. Dräger X-node sensors were installed to measure CO₂ and NH₃ concentration. To determine emissions, a mass balance approach was followed, following the VERA protocol. Ventilation rates were estimated using CO₂ mass balances. CO₂ production was calculated using the formulas of CIGR (2002), based on heat produced by the animals, their estimated weight and the ambient temperature. Initially, the EXP group showed lower weight and feed conversion rate, but these differences disappeared over time. Additional ventilation did not affect growth of the animals. Regarding emissions, the EXP room reduced CO₂ due to better ventilation but increased NH₃ by 40%, due to higher slurry exposure in the solid zone and higher volatilisation. In conclusion, although floor type and ventilation did not significantly influence animal performance, they have a clear effect on air quality. This highlights the need to develop management strategies that balance the reduction of emission and optimise animal production.



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Describing adaptation to heat stress through untargeted metabolomics in pig genetic resources

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Abstract

Climate change is significantly affecting pig production systems worldwide, pushing breeders to face a new challenge: pigs have to respond well to heat-derived stresses while maintaining good productivity. Local pig breeds are genetically and physiologically adapted to their native environments, making them valuable resources for the identification of novel biomarkers. In this study, we used untargeted metabolomics to explore the relationship between the metabolomes of four Italian breeds (Cinta Senese, Mora Romagnola, Italian Large and Italian Duroc) and two Polish breeds or populations (Złotnicka White and Złotnicka White x Złotnicka Spotted) and their response to high temperatures and related stress through the evaluation of the metabolomic profiles over the circadian rhythm of the animals. The study took place in summer, sampling 12 animals per breed at two time points: 1) in the late afternoon (the hottest part of the day) and 2) early in the morning (the coolest part of the day). For each breed, metabolomic profiles of about 700 molecules were compared through a machine learning approach (Random Forest). From each breed, about 30-50 heat-stress-related metabolites were identified and their discriminative power was confirmed with unsupervised analyses. What emerged was a heterogeneous composition of the heat-stress-related metabolome, with few molecules shared between two or more breeds. Nevertheless, this pilot study provided new insights into the biological processes involved in adaptation to heat stress conditions, deepening our understanding of the mechanisms underlying the circadian rhythm physiological processes in these pig breeds. Acknowledgments: This study has received funding from the European Union's Horizon Europe research and innovation programme under grant agreement No. 01059609 (Re-Livestock project).



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The metabolome, a new phenotype: a comparative study of metabolic profiles in plasma and serum

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Abstract

Untargeted metabolomics is increasingly used to characterize complex traits offering a comprehensive view of the physiological state of an animal. Thus, it could serve as a novel phenotype per se, or as additional source of information to improve prediction models. NMR is a technique to infer metabolic content from spectral signal intensities. Plasma and serum are commonly used as substrates in metabolomic, and their composition may vary due to sample processing. The study aims to evaluate if substrate type influences metabolic profiles and explores the potential of raw spectral data to improve profile discrimination. Metabolic profiles of plasma and serum from 15 Holstein (HOL) cows and 15 Avileña-Negra Ibérica (ANI) calves were compared. Two approaches were analyzed: (1) the concentration of 47 metabolites obtained from NMR spectra at 600 MHz and (2) raw spectral intensity data from the NMR for which spectra were preprocessed through frequency calibration, removal of chemical regions, baseline correction, alignment, and normalization. A total of 46,903 chemical shift (ppm) data points were obtained and used as predictor variables. PLS-DA was applied to assess differences between substrates, variable importance in projection (VIP), and correlation analyses. The PLS-DA model showed moderate differences between plasma and serum, more pronounced in HOL than in ANI. VIP values highlighted certain metabolites (9 and 11 for HOL and ANI, respectively) contributing to substrate discrimination. Correlation of correlations between metabolites and substrates were 0.37 and 0.49 for HOL and ANI, respectively. Regarding spectral analysis, PLS-DA showed greater substrate discrimination compared to approach (1), suggesting that raw spectra may capture more subtleties. Plasma and serum metabolic profiles share similarities but there are differences between substrates that are more relevant in HOL than in ANI. Caution needs to be taken when comparing metabolomic profiles from different substrates. The use of raw spectra is a valuable tool to assess differences. Work supported by GLOBALR_WEL (PID2020-113913RR-C31) and HE Re-Livestock.



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