Book of Abstracts of the 8th EAAP International Symposium on Energy and Protein Metabolism and Nutrition (ISEP 2025)

15 – 18 September 2025

Rostock-Warnemünde, Germany

Edited by the Local Organizing Committee of ISEP 2025

The abstracts were peer-reviewed and edited to maintain the scientific standards set by the EAAP International Scientific Committee of ISEP. Contents of all contributions are the responsibility of the authors and do not reflect views of the committees involved in the organization of this symposium.

Organizers of the 8th EAAP International Symposium on Energy and Protein Metabolism and Nutrition (ISEP 2025)

Local Organizing Committee

Cornelia Metges
Harald H. Hammon
Björn Kuhla
Gürbüz Daş

Research Institute for Farm Animal Biology

National Organizing Committee

Ilen Röhe (Universität Rostock)
Jürgen Zentek (Freie Universität Berlin)
Uta Dickhöfer (Christian-Albrechts Universität zu Kiel)

EAAP International Scientific Committee

Luciano Pinotti (President, Italy)
Jacek Skomial (Secretary, Poland)
Mario Luiz Chizzotti (Brasil)
Ignacio Fernandez-Figares (Spain)
Masaya Katsumata (Japan)
James Oltjen (USA)
Daniel Ouellet (Canada)
Jürgen Zentek (Germany)
Leen Vandaele (Belgium)
Cornelia C. Metges (Germany)

Acknowledgement: The following reviewers are gratefully acknowledged for their valuable time and expertise in evaluating the abstracts for this symposium:

G. Bee; G. Daş; U. Dickhoefer; N. Everaert; I. Fernandez-Figares; H. Hammon; M. Katsumata; F. Koch; B. Kuhla; C.C. Metges; J. Oltjen; D. Ouellet; L. Pinotti; H. Reyer; I. Roehe; Q.L. Sciascia; L. Vandaele; J. Zentek

EAAP
European Federation of Animal Science

Energy and Protein
Metabolism and Nutrition

We thank all our ISEP 2025 sponsors

Platinum

Dr. PIEPER
Technologie- und Produktentwicklung
für Landwirtschaft
und
Umwelttechnik

CJ BIO

Gold

EVONIK
Leading Beyond Chemistry

BASF
We create chemistry

iff Danisco Animal Nutrition & Health

Silver

Holm & Laue
passion for calves

H. WILHELM SCHAUMANN STIFTUNG

AMERICAN SOCIETY OF ANIMAL SCIENCE

Bronze

Kaesler Nutrition

DENKAVIT

animal
for scientists, by scientists

CAMPRO SCIENTIFIC

Agilent Technologies

Animal Nutrition
An open-access and peer-reviewed international journal
https://www.sciencedirect.com/journal/animal-nutrition/

We also appreciate the support of the Deutsche Forschungsgemeinschaft

DFG Deutsche Forschungsgemeinschaft
German Research Foundation

Publisher

Research Institute for
Farm Animal Biology

Wilhelm-Stahl-Allee 2, 18196 Dummerstorf, Germany
15. September 2025, Dummerstorf, Germany
https://www.fbn-dummerstorf.de/en/

Citation template: when citing an abstract from this book please use following template:

Author(s) of the abstract (2025). Title of the abstract. In: Book of Abstracts of the 8th EAAP International Symposium on Energy and Protein Metabolism and Nutrition (ISEP 2025), 15–18 September 2025, Rostock-Warnemünde, Germany (p. xx). https://doi.org/10.5281/zenodo.17035972

EDITORIAL

The 8th EAAP International Symposium on Energy and Protein Metabolism and Nutrition (ISEP) took place in Rostock-Warnemünde, from 15 to 18 September 2025.

Twenty-two years after hosting the 1st EAAP International Symposium on Energy and Protein Metabolism and Nutrition in 2003, Rostock-Warnemünde once again welcomed ISEP. Since its inception, the triennial symposium has been held in Vichy (France), Parma (Italy), Sacramento (USA), Krakow (Poland), Belo Horizonte (Brazil), and Granada (Spain).

ISEP 2025, an event organised by the Energy and Protein Metabolism Working Group belonging to the EAAP Nutrition Commission, comprised nine scientific sessions featuring a wide range of lectures and poster presentations on the nutrition and energy, protein and micronutrient metabolism of farmed animals and pets, environmental aspects of animal production, feed science, taste and diet selection and innovative research methods in animal nutrition. Nine distinguished invited speakers and 250 participants from more than 25 countries presented and discussed the latest scientific findings in the field. The symposium was officially opened by the President of the EAAP, Dr. Joel Bérard.

Beyond the scientific programme, the symposium also provided opportunities for networking and exchange, including the Welcome Evening, the Social Dinner, and the Breakfast with Professors for young scientists.

The success of ISEP 2025 was made possible by the contributions of many. The EAAP International Scientific Committee of ISEP, the President of the EAAP Nutrition Commission, Prof. Luciano Pinotti, and numerous collaborators critically revised more than 220 submitted abstracts and contributed to the organization of the scientific sessions. The Local Organizing Committee extends its gratitude to our generous industry sponsors, the German Research Foundation (DFG), our home institution, the Research Institute for Farm Animal Biology (FBN), and our partner universities — Universität Rostock, Freie Universität Berlin, and Christian-Albrechts-Universität zu Kiel — for their support.

We also thank all participants for their scientific contributions, discussions, and engagement, which made the symposium a stimulating and memorable event.

The Local Organizing Committee of ISEP 2025

Cornelia C. Metges, Björn Kuhla, Harald M. Hammon, and Gürbüz Daş

Research Institute for Farm Animal Biology (FBN), Dummerstorf
https://www.fbn-dummerstorf.de/

https://isep2025-fbn.de/

INVITED SPEAKERS

The lectures given by the invited speakers at ISEP 2025 will be published as review articles in a special issue of *animal* in 2026.

Session/lectures	**Date**	**Name**	**Institution**	**Title**
Opening lecture	15.9.2025	Ashild Krogdahl	Norwegian University of Life Sciences (NMBU), Norway	Effects of Antinutrients and Functional Components from Soybeans on Animal Function and Health - Insights from Studies of Monogastric Animals, including Humans and Fish
Protein and energy metabolism in ruminants	15.9.2025	Mike Steele	University of Guelph, Canada	Redefining Energy and Protein Nutrition in the Developing Calf
Protein and energy metabolism in non-ruminants	15.9.2025	Sam Millet	Flanders Research Institute for Agriculture, Fisheries and Food (ILVO), Belgium	Optimizing Health, Welfare and Performance in Newly Weaned Piglets: Finding the Ideal Balance of Amino Acids, Energy, and Protein
Feed processing and additives for sustainable and resilient productivity	16.9.2025	Marta Lourenço	Flanders Research Institute for Agriculture, Fisheries and Food (ILVO), Belgium	Use of Side Streams and By-products as Ingredients in Poultry Feed
Reducing environmental impact of livestock farming	16.9.2025	Ermias Kebreab	University of California, Davis, USA	Ruminants in a Circular Bioeconomy: Nutrition, Methane Mitigation, and Life Cycle Sustainability
Taste, diet selection and behavior in livestock	16.9.2025	Cécile Ginane	French National Research Institute for Agriculture, Food and Environment (INRAE), France	Ethology of Ruminant Feeding: Key Factors influencing Behaviour and Welfare
Nutrient x gene interaction and epigenetics in farm animals	17.9.2025	Maria Siwek	University of Technology and Life Sciences in Bydgoszcz, Poland	Livestock Nutrition and Epigenetics
Methods and technologies for research in the field of sustainable livestock farming	17.9.2025	Martin Beaumont	French National Research Institute for Agriculture, Food and Environment (INRAE), France	From Organoids to Organ-on-chips: Novel in vitro Tools to study Farm Animal Biology
Micronutrient nutrition and metabolism	18.9.2025	Michael Oster	Research Institute for Farm Animal Biology (FBN), Germany	Significance of the Vitamin D system for the Regulation of Mineral Utilization in Pigs and Poultry

Scientific programme

Session 2: Protein and energy metabolism in non-ruminants

Session 3: Reducing environmental impact of livestock farming

Session 4: Nutrient x gene interaction and epigenetics in farm animals

Session 5: Feed processing and additives for sustainable and resilient production

Session 6: Methods and technologies for research in the field of sustainable livestock farming

Session 7: Taste, diet selection and behavior in livestock

Session 8: Micronutrient nutrition and metabolism

Session 9: Fresh from the lab (late breaking abstracts)

Lipidomic profiles of liver and muscle in preweaned calves reveal metabolic advantages of high-fat milk replacer

M.I. Marcondes[1,2], C.R. Ferreira[3], J.A. Torrecilhas[4], N.V. Trópia[5], D.R. Andrade[5]
[1]William H Miner Institute, Chazy, USA. [2]Washington State University, Pullman, USA. [3]Purdue University, West Lafayette, USA. [4]University of Sao Paulo, Botucatu, Brazil. [5]Federal University of Viçosa, Viçosa, Brazil (mmarcondes@whminer.com)

Feeding strategies during preweaning are critical for optimizing metabolic programming, tissue development, and health outcomes in dairy calves. Despite its importance, the impact of fats in milk replacers (MR) on tissue-specific lipid metabolism in neonatal calves remains poorly understood. This study aimed to investigate how varying MR fat content affects the liver and muscle lipidomes of preweaned calves. A total of 55 Holstein-Friesian male calves were enrolled at birth in a controlled feeding trial and assigned to one of two treatment groups: a high-fat MR (30% fat) or a low-fat MR (15% fat), each fed at 8 L/day. The MR were comparable in terms of protein and minerals, but the high-gain MR had higher energy. Five calves were harvested at the beginning of the trial to serve as the reference group. All animals were individually housed and monitored throughout the trial for health status, performance, and feed intake. While the entire cohort was used for assessing growth, intake, and clinical parameters, lipidomic profiling focused on a subset of animals ($n = 7$ per treatment) slaughtered at 30 days of age, allowing for the evaluation of early-life lipid metabolic adaptations. Tissue samples from liver and skeletal muscle were analyzed using multiple reaction monitoring (MRM) profiling mass spectrometry. In liver, lipid extracts were assessed for over 3,700 transitions, leading to the detection of 1,294 lipids across 11 major classes. MR did not affect performance ($P < 0.05$). In liver tissue, calves fed the high-fat MR showed elevated levels of acylcarnitines (e.g., CAR(18:1), CAR(5:1)), phosphatidylcholines (e.g., PC(34:2)), and triacylglycerides (e.g., TG(46:2)_C18:2), suggesting increased lipid turnover, enhanced fatty acid oxidation, and improved energy handling. Lipid ontology analysis further revealed significant enrichment in pathways associated with lipid droplet formation, mitochondrial membrane remodeling, and fatty acid utilization. In contrast, the low-fat group exhibited increased ceramides and cholesteryl esters—lipids often associated with inflammatory signaling, impaired lipid clearance, and hepatic stress (Li et al., 2014). In muscle, high-fat-fed calves presented greater abundance of phosphatidylcholines (e.g., PC(38:3), PC(40:5)) and triacylglycerides (e.g., TG(42:2)_C14:1), indicative of improved energy storage capacity and membrane flexibility. These changes align with a phenotype favoring muscular endurance and metabolic resilience. On the other hand, the low-fat MR group had higher concentrations of sphingomyelins and storage lipids linked to membrane rigidity and stress responses (Xie et al., 2021). Thus, a high-fat MR supports a more favorable lipidomic profile in liver and muscle, characterized by efficient lipid utilization, reduced metabolic stress, and better adaptation to early postnatal life. Modulating tissue-specific lipid pathways through nutrition underscores the potential of lipidomics to guide precision feeding strategies that improve calf health, productivity, and long-term metabolic outcomes in the dairy industry.

References

Li, M., Butka, E., Wang, X., 2014. Scientific Reports 4, 6581. https://doi.org/10.1038/srep06581
Xie, Z., Ferreira, C.R., Virequ, A.A., Cooks, R.G., 2021. Chemistry and Physics of Lipids 235, 105048. https://doi.org/10.1016/j.chemphyslip.2021.105048

Funding

This study was supported by Trouw Nutrition.

Exchanging milk replacer for solid feed on a paired gain basis alters circadian patterns but not daily energy expenditure in veal calves

A.J.W. Mens, M.S. Gilbert, W.J.J. Gerrits
Wageningen University & Research, Wageningen, Netherlands (myrthe.gilbert@wur.nl)

Veal calf welfare and economic perspective are important incentives to exchange part of the milk replacer (MR) by solid feed. This changes nutrient profiles from dairy proteins, lactose and long-chain fatty acids towards microbial proteins and short-chain fatty acids. To test whether this changes energy expenditure and the composition of BW gain, we quantified the effects of increasing the intake of solid feed (SF) on protein and fat deposition in pair-housed calves. Twenty-four pairs of male Holstein-Friesian calves (68±7.7 kg) were assigned to one of four dietary treatments, supplying either 20 g/kg BW$^{0.75}$/d (LowSF), 30 g/kg BW$^{0.75/}$d (MiddleSF), or 40 g/kg BW$^{0.75}$/d (HighSF or Hay) of solid feeds. The solid feed consisted of concentrate and chopped wheat straw in a 90:10 ratio (LowSF, MiddleSF and HighSF), or of concentrate and long hay in a ratio of 70:30 as fed (Hay). The quantity of MR was fixed for HighSF at 39 g/kg BW$^{0.75}$/d and the amount of MR for the other treatments was based on a paired-gain strategy to achieve comparable BW gain across treatments during a 5-week adaptation phase. Complete energy and nitrogen balances of pairs of calves were measured using indirect calorimetry and physical activity was recorded continuously by a radar device. To achieve similar BW gains, MR intakes of the LowSF, MiddleSF and Hay treatment averaged 50.3, 45.3 and 42.1 g DM/kg BW$^{0.75}$/d, respectively. Metabolizable energy intake (1275±292 kJ/kg BW$^{0.75}$/d), energy retention (545±187 kJ/kg BW$^{0.75}$/d), protein (177±14 kJ/kg BW$^{0.75}$/d) and fat deposition (367±368 kJ/kg BW$^{0.75}$/d) were not affected by treatment. Heat production tended to be increased (P=0.10) for hay-fed calves compared to straw-fed calves. Methane production increased (P<0.001) with SF intake, being lowest for LowSF (13.1 kJ/kg BW$^{0.75}$/d) and highest for Hay (26.7 kJ/kg BW$^{0.75}$/d). Daily activity-related heat production (84±19 kJ/kg BW$^{0.75}$/d) and resting metabolic rate (RMR; 648±88 kJ/kg BW$^{0.75}$/d) were not affected by treatment. However, within-day patterns of RMR exhibited clear treatment-dependent patterns. During the night, RMR was lower for LowSF and MiddleSF than for HighSF and Hay, indicating an increase in nutrient absorption from rumen fermentation, but also reflecting an increase in metabolic processes related to rumen development. During the day, the response in RMR to the MR meals were proportional to the size of the MR meal. In this study, calves were group-housed which could reduce abnormal behaviors and subsequent effects on energy expenditure compared to individually housed calves as often done for indirect calorimetry measurements. In conclusion, exchanging milk replacer for solid feeds in a paired-gain setting does not affect protein and fat deposition, despite sizeable differences in the within-day patterns of metabolism.

Funding

This research was conducted within the framework of the public private partnership "TKI-AF-12064, Duurzame verbreding grondstoffenpakket voor vleeskalveren," and financially supported by the Ministry of Agriculture, Nature and Food Quality, and Stichting Brancheorganisatie Kalversector, the Netherlands.

Concentrations and excretions of proteins, nitrogen compounds and purine derivatives in the urine of neonatal brown swiss calves

A.M. Vorndran[1], S. Kehraus[2], J. Steinhoff-Wagner[1]
[1]Professorship of Animal Nutrition and Metabolism, Technical University of Munich, 85354, Freising, Germany. [2]Institute of Animal Science, University of Bonn, 53115, Bonn, Germany (jsw@tum.de)

Urine, as an excretory substance from various detoxification pathways, allows for the identification of biomarkers to diagnose diseases, assess metabolic status, and evaluate microbial activity in the rumen. The composition of urine can provide critical insights into the physiological adaptations of neonatal calves. However, data on neonatal calves is lacking, particularly regarding a feedstuff change during the first week of life. We hypothesized that there would be significant differences in urine constituents after a change in feedstuff from transition milk to milk replacer compared to continued feeding of milk. In this study, eighteen calves were housed individually in calf hutches and fed their dam's colostrum initially, followed by transition milk twice daily until the sixth feeding. At the seventh feeding, calves were randomly assigned, but distributed by sex, to either milk or milk replacer for the remaining eight feedings. The animals were fed twice daily in unlimited amounts from initially bottles and then nipple buckets. The calf hutches were equipped with urine trays for quantitative urine collection throughout the first week. Urine was collected twice daily before feeding. Laboratory analyses included dry matter, density, nitrogen, urea, protein, creatinine, hippuric acid, purine derivatives, minerals, electrical conductivity, salinity, and brix value. Data were analysed using a mixed model ANOVA in RStudio with sex and treatment as fixed and calf ID as random factor. All analytical values were significantly influenced by time, showing decreasing concentrations from birth to day 7. Proteinuria was observed in all calves after colostrum ingestion but disappeared by the third day. Allantoin was the most abundant purine derivative, indicating a sufficient purine degradation during early life. Calves fed milk replacer exhibited significantly higher mineral concentrations, excretions, electrical conductivity, and salinity in urine compared to milk-fed calves, despite similar feed intake levels. No significant differences were found in nitrogen and urea concentrations between treatments, however their concentration in urinary DM significantly decreased upon feedstuff change as a result of the increase in mineral excretion. Significant changes in urine composition occur within hours after birth as neonatal calves adapt to their environment. Initial proteinuria develops and rapidly disappears. Increased feed consumption leads to higher water content in urine, reducing constituent concentrations. Feed type failed to affect urinary nitrogen or urea concentrations, despite differences in protein and energy intake. High purine derivative concentrations immediately after birth suggest elevated nucleic acid turnover in neonatal calves.

Energetic evaluation of a lipopolysaccharide challenge in growing beef steers

L.G. Canterbury[1], P.R. Broadway[2], C.W. Dornbach[1], K.D. Childress[1], M.S. Grant[1], M.G. Baker[1], N.C. Burdick Sanchez[2], M.M. Smithyman[3], M.L. Galyean[1], K.E. Hales[1]
[1]Texas Tech University, Lubbock, USA. [2]USDA-ARS, Lubbock, USA. [3]New Mexico State University, Las Cruces, USA (kristin.hales@ttu.edu)

Available energy in cattle is important for immune activation, the acute-phase response, and for combatting and recovery from an infection. Kvidera et al. (2016) noted that cattle can use more than 1 kg of glucose within 12 h after being challenged with lipopolysaccharide (LPS), which induces an inflammatory response, and the acute-phase response can be replicated to simulate a diseased state (Smock et al., 2023). Failure to consume adequate energy during this time could limit the ability of the immune system to fight off a pathogen or virus, thereby extending the duration of an immune challenge or worsening the negative effects. We evaluated the whole-body energetic cost of an activated immune system after a lipopolysaccharide challenge. Growing beef steers (n = 6; BW = 273 kg ± 25.2) were used in a randomized complete block design with steers as the experimental unit. Period (PRE- or POST-lipopolysaccharide [LPS]) was included in the model as a fixed effect and steer as a random effect. Steers were fasted 48 h before indirect respiration calorimetry measurements, after which they were placed in stalls, and their heads were secured into portable headboxes for gas (O_2, CH_4, CO_2) exchange collections before the LPS challenge (PRE-LPS). After 24 h in the headboxes, gas collection bags were changed, and LPS was administered (0.20 µg of LPS/kg of BW) through a jugular catheter. After LPS administration, another 24-h gas exchange was obtained (POST-LPS). Blood samples were collected for analyses of complete blood count, serum chemistry, and blood glucose determination. Fasted heat production did not differ between PRE and POST-LPS periods ($P \geq 0.36$). Oxygen consumption did not differ between periods ($P \geq 0.43$), whereas methane production decreased ($P \leq 0.01$) from PRE to POST-LPS, as the fast was extended. After the LPS challenge, circulating white blood cells decreased by 83% ($P = 0.01$), neutrophils decreased by 94% ($P = 0.01$), and lymphocytes decreased by 75% ($P = 0.01$). Blood glucose decreased 65% ($P = 0.01$) between 1 and 4 h POST-LPS administration. Differences among blood variables indicate that an acute immune response occurred because of LPS administration. It is likely that the small number (n = 6) of steers in this pilot study precluded our ability to detect a difference in heat production before and after an intravenous LPS challenge. Nonetheless, the approach used should provide a basis for additional research to more clearly define the effects of an activated immune system on whole-body energetics.

References

Kvidera, S.K., Horst, E.A., Abuajamieh, M., Mayorga, E.J., Sanz Fernandez, M.V., Baumgard, L.H., 2016. Journal of Animal Science 94, 4591-4599. https://doi.org/10.2527/jas.2016-0765

Smock, T.M., Broadway, P.R., Burdick Sanchez, N.C., Carroll, J.A., Theurer, M.E., Hales, K.E., 2023. Journal of Animal Science 101, skad133. https://doi.org/10.1093/jas/skad133

Funding

Research was supported by the Thornton Endowment in Animal Science at Texas Tech University.

Effects of supplementing rumen-protected branched-chain amino acids in a low protein diet on nitrogen deposition and nitrogen metabolism in growing beef cattle

S.Y. Xu, G.Y. Zhao, M.M. Li
China Agricultural University, Beijing, China (limeng2021@cau.edu.cn)

Beef cattle exhibit low efficiency (around 25%) in utilizing dietary nitrogen, and using low protein diets helps conserving protein resources and reduce nitrogen emissions but compromises production performance (Shen et al. 2024). This study aimed to investigate the effects of supplementing rumen-protected branched-chain amino acids (RP-BCAA) in a low protein diet on growth performance, ruminal fermentation, nutrient digestibility, and nitrogen metabolism in growing beef cattle. Eight healthy Simmental crossbred bulls (6 – 8 months of age, initial BW 238.44 ± 10.31 kg) were selected and assigned to a replicated 4 × 4 Latin square design. The four treatments were (1) a high protein diet (HCP, CP = 14.62%), (2) a low CP diet (LCP, CP = 10.40%), (3) RP-LM diet: LP diet supplemented with 32 g/d of rumen-protected lysine and methionine (Lys : Met = 3:1；bioavailability = 36%), and (4) RP-BCAA diet: LP diet supplemented with 64 g/d of RP-BCAA (Leu : Ile : Val = 2:1:1；bioavailability = 23%). The experiment comprised 4 periods, each lasting 20 days with a 15 days for adaptation and 5 days for sampling. No significant differences were observed in dry matter intake among treatments ($P = 0.72$). Compared with the LCP, both the HCP and RP-BCAA groups exhibited significantly increased average daily gain (1.07 kg/d v.s. 1.52 and 1.39 kg/d; $P < 0.001$) and feed efficiency (12.8% v.s. 18.2% and 17.0%; $P = 0.001$). Compared to the HCP, LCP, RP-BCAA, and RP-LM decreased ruminal microbial protein, NH_3N, isobutyrate, valerate, and isovalerate concentrations ($P < 0.001$), as well as total tract protein digestibility ($P < 0.001$). These treatments also reduced plasma urea N concentration, total nitrogen excretion, urinary nitrogen, and the proportion of urinary nitrogen in total excretion ($P < 0.001$), without affecting fecal nitrogen excretion. Furthermore, HCP, RP-BCAA, RP-LM groups showed significantly greater nitrogen deposition (29.1 g/d v.s. 55.4, 39.8, and 40.0 g/d) and nitrogen utilization efficiency relative to the LCP (21.6% v.s. 28.1%, 27.6%, and 29.0%; $P = 0.001$). These findings suggest that supplementing RP-BCAA in LP diets can improve growth performance and nitrogen retention while reducing nitrogen excretion in growing beef cattle.

Reference

Shen, C., Wang, J., Zhao, G., Li, M.M., 2023. Animal Feed Science and Technology 302, 115670. https://doi.org/10.1016/j.anifeedsci.2023.115670

Funding

This work was funded by National Key R&D Program of China (2023YFD1301405-4).

Duodenal supply and small-intestinal digestibility of amino acids from pulp silage fed to lactating dairy cows

S.E. Räisänen, N.P. Hansen, S.K. Jensen, M.R. Weisbjerg
Department of Animal and Veterinary Sciences, Research Centre Foulum, Aarhus University, Tjele, Denmark (susanna.raisanen@anivet.au.dk)

This study aimed to assess the supply and small-intestinal digestibility (SID) of amino acids (AA) from grass silage (GS) or processed pulp silage from a biorefinery pressed once (1P) or twice (2P) of two developmental stages (Dev), i.e., early (E) and late (L; 35 and 44 d of regrowth, respectively). Six lactating multiparous Holstein cows with ruminal, duodenal, and ileal cannulas (energy-corrected milk 30.7 ± 3.7 kg/d, dry matter intake (DMI) 20.6 ± 2.6 kg/d, and days-in-milk 176 ± 93 d) were enrolled in the study. The experimental design was a 6x4 incomplete Latin square with four, 21-day periods and a 2x3 factorial arrangement of treatments. The two pulps were ensiled similar to GS resulting in three silages within each Dev: E-GS, E-1P, E-2P, L-GS, L-1P, and L-2P. Treatments were fed as TMR including (DM-basis) an experimental silage (65.0%), soybean meal (6.82%), rolled wheat (26.4%), and mineral mix (1.82%). A total of 12 samples (covering every 2 h of a 24-h day) were collected from duodenal and ileal digesta, and one rumen fluid sample for microbial pellet to determine purine and AA composition. Data were analysed with the '*lmer*' function in R, with Dev and processing, their interaction, and period as fixed and cow as random effects. Silage nutrient composition, total AA content, and N-fractions have been previously reported in Hansen et al. (2023). Duodenal flow of microbial AA was lower ($P \leq 0.05$) for 1P compared with GS (49.6 vs. 56.0 g/kg DMI), regardless of Dev. Similarly, of individual AA, Arg, Gly, Leu, Pro, Ser, and Val from microbial AA (g/kg DMI) were 13.1 to 14.5% lower ($P \leq 0.05$) for 1P vs. GS. There was an interaction ($P \leq 0.04$) between Dev and processing for duodenal flow of feed AA (g/kg DMI), except for Cys and Gly, being greatest ($P \leq 0.05$) for E-2P followed by E-1P, L-2P, L-1P and E-GS, and was lowest for L-GS. There was no difference in SID for total AA across treatments (average 77.9%; SEM = 1.11), and SID varied between individual AA [average across treatments: 65% (SEM = 4.0) for Cys and 85% (SEM = 2.7) for Gly]. However, SID increased by 2.3 and 2.6 %-units ($P \leq 0.05$) with 2P vs. GS for Glu and Lys, increased with 1P and 2P for Met and Pro (5.9 and 3.0 %-units, respectively), and tended to ($P < 0.08$) increase for Ala and Ile. The greater duodenal flow, and improved SID, of pulp silages may stem from the greater availability of fibre-bound AA in the small intestine. Based on data from this experiment, SID varies notably between individual AA, and the supply of digestible AA from perennial ryegrass was improved when fed as pulp silage.

Reference

Hansen, N.P., Jensen, S.K., Johansen, M., Hellwing, A.L.F., Ambye-Jensen, M., Larsen, M., Weisbjerg, M.R., 2023. Journal of Dairy Science 106, 937-953. https://doi.org/10.3168/jds.2022-21851

The relation between nitrogen efficiency and nitrogen partitioning in dairy cows on commercial farms

M.G.J. Edens[1], E.L.P. Guennoc[2], M.S. Gilbert[1], H. Bovenhuis[2], J. Dijkstra[1]
[1]Animal Nutrition Group, Wageningen University & Research, Wageningen, Netherlands. [2]Animal Breeding and Genomics Group, Wageningen University & Research, Wageningen, Netherlands (margreet.edens@wur.nl)

Enhancing nitrogen (N) use efficiency (NUE; milk N output/N intake) improves resource efficiency and reduces the environmental impact of dairy farming. Increasing the faecal-to-urinary N ratio (FN:UN) is desirable, as faecal N is less prone to volatilization, denitrification, and leaching than urinary N. This study aimed to improve our understanding of NUE variability in dairy cows by collecting and analysing faecal and urinary samples of 483 cows across four Dutch commercial farms, recording individual feed intake. Three farms were visited twice (different cows were sampled during each visit to avoid repeated measures), and one farm was visited once, resulting in seven sampling periods. During each period, milk yield and composition were determined, and spot samples of faeces and urine were collected at six 4-hour intervals over three to four consecutive days, covering all 4-hour periods within a 24-hour cycle. Nitrogen content was analysed in pooled urine, faeces, and feed. Creatinine and acid-insoluble ash concentrations were used to estimate urine and faecal output, respectively. Microbial N production was estimated using purine derivative excretion in urine. Data were analysed using a mixed model, with NUE as dependent variable and N partitioning, sampling period, and the interaction with sampling period as explanatory variables. Days in milk (DIM), body weight and parity were included as covariates, and period was included as random effect. On average, cows had a parity of 1.5±0.73, were 138±53.2 DIM, producing 37.4±7.33 kg/day of milk with a NUE of 305±42.6 g/kg, apparent total tract N digestibility of 65.6±6.67%, microbial N efficiency of 28.6±5.34 g/kg digested organic matter, FN:UN of 1.06±0.249, urinary N/N intake of 325±53.1 g/kg, urinary N/digested N of 502±105.7 g/kg, and N retention of 23 ±67.3 g/d. Dietary crude protein averaged 181±9.7 g/kg DM. Preliminary results indicate that NUE related positively with microbial N efficiency and negatively with N retention ($P<0.01$). Furthermore, NUE related positively with N digestibility (N digestibility × period, $P<0.05$) and negatively with FN:UN (FN:UN × period, $P<0.05$) in three out of seven sampling periods. Additionally, NUE was positively related with the urinary N to N intake ratio (urinary N/N intake × period, $P<0.01$) and urinary N to digested N ratio (urinary N/digested N × period, $P<0.01$) in two out of seven sampling periods. In conclusion, preliminary findings reveal that an increase in NUE is consistently associated with greater microbial N efficiency and lower N retention. An increase in NUE was associated with a shift in N excretion, with a lower proportion of N excreted in faeces and a higher proportion excreted in urine, depending on farm or period of visiting farms.

Funding
This study is part of the SYNERGIA project, organized by Wageningen University and Research in cooperation with Next Food Collective and Universities of Delft, Twente, Eindhoven, and Nijmegen. Funding for this research was obtained from the Netherlands Organisation for Scientific Research (NWO grant 17626), IMEC-One Planet and other private parties.

Dietary induced changes in the absorption rate of volatile fatty acids in Holstein cows

M.H. Kjeldsen, M.R. Weisbjerg, P. Lund, M. Larsen
Aarhus University, Viborg, Denmark (Maria.kjeldsen@anivet.au.dk)

The aim of the current study was to quantify the effect of changes in diet on absorption rate of volatile fatty acids (VFA) in the rumen using the “washed rumen” procedure. Eight lactating rumen cannulated 2nd parity Holstein cows were fed one of two diets with either low forage proportion (35% of DM, F35) or high forage proportion (63% of DM, F63). In total, three buffers were mixed: a control buffer without VFA added (0-BUF), a buffer with an acetate and propionate concentration of 70 and 20 mmol/kg, respectively (AC), and a buffer with an acetate and propionate concentration of 50 and 40 mmol/kg, respectively (PR). Additionally, 2, 10, and 3 mmol/kg for isobutyric, butyric, and valeric acid, respectively, were added in both the AC and PR buffers. The composition of minerals were similar to the control buffer in the study by Storm et al. (2011). Before the rumen evacuation, access to feed and drinking water was removed. The rumen was temporarily emptied, first rinsed with lukewarm water followed by three rinses with 10 kg saline/rinse (0.9% NaCl). Hereafter, 20 kg 0-BUF was allocated and removed after 15 min, before allocating either the AC or the PR buffer. All cows tried both buffers in a randomized order. Samples were taken from the rumen 3, 9, 20, and 30 min after buffer allocation. The rinsing procedure with saline and 0-BUF was repeated before allocation of the other buffer with VFAs and repeated sampling. The VFA concentrations were measured by gas chromatography. Absorption rates (%/h) of acetate, propionate, and butyrate were estimated by linear regression of the natural log-transformed VFA concentration against time in the rumen. The estimated absorption rates were statistically analyzed using a lmer-model (nlme R-package) with diet, buffer and their interaction as fixed effects, and cow as random effect. The absorption rate of propionate tended to be higher in F35 fed cows with the PR buffer compared with other diet by buffer combinations (77.7 vs. 64.1%/h ± 5.4; P=0.08 for the interaction between diet and buffer). The results emphasize that the absorption rate and concentration of propionate are positively correlated. Furthermore, there is an indication that the absorption rate of propionate is linked to the dietary priming of the rumen epithelium, as cows fed a diet with low forage: concentrate ratio had a greater propionate absorption rate. Absorption rates of acetate and butyrate were not affected by diet or buffer ($P \geq 0.26$) and averaged 52.3 ± 3.76%/h and 65.6 ± 2.47%/h, respectively. Potential correlation between absorption rate and methane phenotypes is to be invested.

Reference

Storm, A.C., Hanigan, M.D., Kristensen, N.B., 2011. Journal of Dairy Science 94, 3980-3994. https://doi.org/10.3168/jds.2010-4091

Associations between serum amino acids, non-esterified fatty acids and β-hydroxybutyrate in dairy cows prone to hyperketonaemia

M.O. Matthäi[1], B. Kuhla[2], G. Köller[3], S.U. Kononov[1], J. Rehage[4], M.R. Wilkens[1]
[1]Institute of Animal Nutrition, Nutrition Diseases and Dietetics, Leipzig University, Leipzig, Germany. [2]Research Institute for Farm Animal Biology (FBN), Dummerstorf, Germany. [3]Large Animal Clinic Laboratory, Leipzig University, Leipzig, Germany. [4]Clinic for Cattle, University of Veterinary Medicine Hannover, Foundation, Hanover, Germany (mirja.wilkens@uni-leipzig.de)

At the onset of lactation, energy requirements in dairy cows exceed energy supply resulting in a mobilisation of fat and muscle tissue. Hyperketonaemia [HK] develops when acetyl-CoA, mainly derived from non-esterified fatty acids [NEFA] cannot be further metabolised within the citrate cycle. As glucogenic amino acids can be used to replenish the citrate cycle, we investigated the associations between the amino acid profile and HK in dairy cows. Samples were derived from cows of two dairy herds that had been monitored due to a known increased risk for ketosis. Animals had been sampled on a regular basis (1 days in milk [DIM], 8 DIM, 16 DIM, 24 DIM, and 32 DIM) and treated with oral propylene glycol (300 mL) if serum beta-hydroxybutyrate [BHB] was > 1.2 mM (group HK1) and intravenous glucose monohydrat (220 g) and fructose (200 g) if BHB was > 1.7 mM (group HK2). The latter ones were sampled again after 24 h and treated again if necessary. The remaining serum from sampling was analysed for NEFA and a subset of 25 samples stratified for herd and occurrence of hyperketonaemia was additionally analysed for free amino acids by means of HPLC at 8 DIM. Data of the different groups (HK1, n=11; HK2, n=6; non-ketotic, NHK, n=8) were analysed using 1way ANOVA with Dunnett´s post-test. Correlations between serum parameters were calculated according to Pearson. Generally, 3-methylhistidine at 8 DIM was correlated with NEFA at 8 DIM (r: 0.55), reflecting the link between mobilisation of both, muscle and fat tissue. Glycine and leucine were positively associated with NEFA at 8 DIM, especially in HK cows (r: 0.83 and 0.68, resp.), while a positive relationship between methionine and NEFA could be observed exclusively in NHK controls (r: 0.78). It might be speculated that the amino acid profile in early lactation is also linked with metabolic stability later in lactation. For instance, isoleucine at 8 DIM was correlated with BHB at 32 DIM exclusively in animals that developed HK > 8 DIM (r: 0.96) and the ratio between BHB and NEFA at 32 DIM probably reflecting the efficiency of energy utilisation from fat tissue mobilisation was inversely correlated with tyrosine at 8 DIM when all cows were considered (r: -0.54). In addition, animals that received treatment more than once (HK2) displayed significantly lower concentrations of glutamate at 8 DIM (68.2 ± 14.3 µM) in comparison to NHK controls (108 ± 29.5 µM) or HK1 cows (HK1, 87.0 ± 14.7 µM). For β-alanine, a precursor of carnosine, we found higher concentrations in HK2 cows (3.98 ± 0.56 µM) in comparison to NHK controls (2.56 ± 0.63 µM), suggesting altered muscle metabolism in HK2 cows.

Production responses to metabolizable protein supply did not differ between Holstein and Ayrshire cows

D.R. Ouellet[1], C. Benchaar[1], F. Hassanat[2], C. Cortes[3], H. Lapierre[1]
[1]Agriculture and Ari-Food Canada, Sherbrooke, QC, Canada. [2]Agriculture and Ari-Food Canada, Quebec, QC, Canada. [3]Agrinova, Alma, QC, Canada (daniel.ouellet@agr.gc.ca)

The aim of this trial was to determine whether the NRC (2001) feeding evaluation system developed mainly from Holstein (HO) data is applicable to a smaller breed as Ayrshire (AY), by comparing their responses to incremental supply of metabolizable protein (MP). Six HO and 6 AY were used in 2 replicated 3×3 Latin squares (21-d periods). Cows were fed diets formulated to supply 85, 100, or 115% of their MP requirements and 100% of their energy requirement (NRC, 2001). To reach this objective, because of difference in dry matter intake (DMI) between breeds relative to milk protein yield (MPY), dietary CP concentrations were lower for HO (13.9, 16.7 and 17.6%) than for AY (15.3, 17.1 and 18.7%). The data from d 17 to d 21 were used for statistics. Effect of breed, MP supply, and breed´MP interaction were assessed using MIXED procedure of SAS, with polynomial contrasts to determine the linear and quadratic effects of MP supply. Data are presented for 85, 100 and 115% MP, for HO vs. AY, respectively. No breed´MP interaction (P=0.14) were observed. The DMI was greater (P=0.01) for HO vs. AY (25.6, 25.9 and 25.8 vs. 21.6, 22.2 and 21.9 kg/d; SEM=0.64), but not affected by MP supply. Milk yield was greater (P=0.01) for HO vs. AY (42.2, 42.9 and 42.7 vs. 34.5, 36.2 and 35.9 kg/d; SEM=1.20) but also not affected by MP supply (P>0.10). The MPY tended to be greater (P=0.07) for HO vs. AY with MP supply having linear and quadratic effects (P≤0.05; 1.18, 1.26 and 1.23 vs. 1.04, 1.12 and 1.10 kg/d; SEM=0.052). The tendency (P=0.07) for a lower milk urea observed for HO (8.2, 13.3, and 15.1, vs. 10.4, 14.5 and 17.2 mg/dL; SEM=0.67) might be related to their lower MP balance compared with AY. The N efficiency linearly declined (P≤0.001) with increasing MP supply, and was similar between breeds (33.2, 30.7, and 29.0, vs. 33.0, 30.6, and 28.7%; SEM=0.77). In conclusion, AY cows responded to incremental MP supply similarly to HO cows, with no increment of MPY when fed above 100% of their estimated MP requirements. Therefore, a feed evaluation system such as NRC (2001) developed mainly based on HO data is applicable to a smaller breed like AY, as long as MP are used to balance the diets and not CP.

Reference

NRC, 2001. Nutrient Requirements of Dairy Cattle. 7th rev. ed. The National Academies Press. Washington, DC.

Funding

The authors are grateful for the funding support of Ayrshire Canada and Ayrshire Québec (St-Hyacinthe, QC, Canada), Agrinova (Alma, QC, Canada), and Agriculture and Agri-Food Canada (Ottawa, ON, Canada).

Effect of metabolizable protein supply on leucine kinetics in Holstein and Ayrshire cows

D.R. Ouellet[1], C. Benchaar[1], F. Hassanat[2], C. Cortes[3], H. Lapierre[1]
[1]Agriculture and Agri-Food Canada, Sherbrooke, QC, Canada. [2]Agriculture and Agri-Food Canada, Quebec, QC, Canada. [3]Agrinova, Alma, QC, Canada (daniel.ouellet@agr.gc.ca)

The objective of this trial was to compare the effect of metabolizable (MP) supply on whole body Leu kinetics in Holstein (HO) and Ayrshire (AY) cows. Six HO (704 kg BW) and 6 AY (602 kg BW) were used in 2 replicated 3×3 Latin squares (21-d periods). Diets were formulated to supply 85%, 100% and 115% of MP requirements, based on the milk protein yield (MPY) of each breed (NRC, 2001). On d 18 or 20, [^{13}C]Na-bicarbonate (1.8 mmol/h, 99% ape), and on d 19 or 21, L[1-^{13}C]Leu (3.9 mmol/h, 99% ape) were infused into one jugular vein for 4 h, in 6 cows each day. During the infusions, 5 blood samples were taken from the contralateral jugular vein every 30 min for the last 2 h of infusions. The isotopic enrichments of blood CO_2 and plasma Leu were determined to calculate Leu kinetics using a 2-pool model. The effects of breed, MP supply, and breed×MP interaction were evaluated using MIXED procedure of SAS, with polynomial contrasts to determine the linear and quadratic effects of MP supply. Data are presented for 85, 100, and 115% MP, for HO vs. AY, respectively. The HO effectively consumed 81, 100 and 113%, and the AY, 88, 104 and 121% of their MP requirements. Absolute values of Leu whole body rate of appearance (Leu_Ra: 119, 130 and 140 vs. 101, 114 and 126 mmol/h, SEM=3.2), and Leu used for protein synthesis (Leu_PS: 107, 112 and 114 vs. 92, 94 and 95 mmol/h, SEM=2.5) were greater ($P \leq 0.001$) for HO vs. AY, with no breed×MP interaction. However, Leu_Ra per unit of metabolic BW did not differ between breed. There was a breed×MP interaction ($P \leq 0.02$) on Leu oxidation (12, 18 and 26 vs. 10, 21 and 30 mmol/h; SEM=1.5) and on fractional Leu oxidation, which increased less rapidly in HO than in AY with increasing MP supply. This might be related to the experimentally smaller MP supply relative to requirements in HO than in AY. Leu secreted in milk protein tended to be higher in HO vs. AY ($P=0.07$; 39, 42 and 41 vs. 35, 37 and 37 mmol/h; SEM=1.7). However, there was no effect of breed and no breed×MP interaction on the proportion of Leu in milk relative to Leu_PS and Leu from protein degradation relative to Leu_PS. Overall, increasing MP supply increased ($P \leq 0.01$; linear) Leu_Ra, and Leu_PS, and altered ($P \leq 0.04$; linear and quadratic) Leu in milk protein, as mentioned previously with no interaction with breed. This indicates that Leu kinetics and associated protein metabolism would respond similarly to increased MP supply in HO and in AY cows.

Reference

NRC. 2001. Nutrient Requirements of Dairy Cattle. 7th rev. ed. The National Academies Press. Washington, DC.

Funding

The authors are grateful for the funding support of Ayrshire Canada and Ayrshire Québec (St-Hyacinthe, QC, Canada), Agrinova (Alma, QC, Canada), and Agriculture and Agri-Food Canada (Ottawa, ON, Canada).

Energy balance and net energy for maintenance of beef x dairy crossbred steers

L.G. Canterbury[1], M.L. Galyean[2], C.W. Dornbach[1], K.D. Childress[1], A.C. Thompson-Smith[1], B.J. Sitka[1], T.A. Loeffler[1], M.S. Grant[1], M.G. Baker[1], N.C. Burdick Sanchez[3], P.R. Broadway[3], K.E. Hales[1]
[1]Department of Animal and Food Sciences, Texas Tech University, Lubbock, USA. [2]Department of Veterinary Sciences, Texas Tech University, Lubbock, USA. [3]United States Department of Agriculture, Agriculture Research Service, Livestock Issues Research Unit, Lubbock, USA (Kristin.Hales@ttu.edu)

Our objective was to determine the partitioning of energy in beef × dairy crossbred steers fed at 2 multiples of maintenance and to determine their net energy for maintenance (NE_m) requirement. Beef × dairy crossbred cattle make up approximately 28% of the cattle on feed in the United States. Their NE_m requirement and efficiency of metabolizable energy use is currently unknown but is hypothesized to be intermediate between beef steers and dairy steers. Seventeen beef × dairy steers with Holstein dams and Angus or Angus × Simmental sires were used in a crossover design (initial BW = 492 ± 21 kg). Steers were assigned randomly to either maintenance energy intake (1X; estimated at 84 kcal/kg of metabolic body size) or 2-times maintenance energy intake (2X). Total fecal and urine collections were obtained over 6 d along with gas exchange over a 24-h period. After the collection period, steers were fasted, and fasting heat production (FHP) estimates were obtained using indirect respiration calorimeter headboxes (24 h). Body weight and DMI differed ($P < 0.01$) between treatments. On a megacalorie basis, digestible energy, fecal and urinary energy loss, metabolizable energy, heat production, and recovered energy were greater ($P < 0.01$) for the 2X steers. Total methane production tended ($P = 0.06$) to increase for 2X vs. 1X steers. Apparent DM digestibility was less ($P = 0.02$) in 1X than in 2X steers. As a percentage of intake energy (IE), urinary energy loss, metabolizable energy, heat production, and retained energy were greater ($P = 0.02$) for 2X than for 1X steers. Fecal energy loss tended to increase ($P = 0.07$) for 2X steers, which caused a tendency ($P = 0.07$) for digestible energy, as a percentage of IE, to decrease for 2X vs. 1X steers. As expected, enteric methane production, as a percentage of IE, was greater ($P < 0.01$) for 1X steers. Using metabolizable energy intake and recovered energy during FHP, 1X, and 2X measurements, the estimate of NE_m was 117 kcal/kg $BW^{0.75}$. Steers were outside of thermoneutral conditions for 41% of the study and were younger than the beef and dairy steers evaluated for previous NE_m determinations (Blaxter and Wainman, 1966), both of which could have contributed to an increased NE_m. The NE_m requirement of beef x dairy steers deserves further research to determine whether the value we observed is repeatable and to directly compare it with purebred beef and dairy cattle in the United States.

Reference

Blaxter, K.L., Wainman, F.W., 1966. British Journal of Nutrition 20, 103-111. https://doi.org/10.1079/BJN19660012

Funding

Research was supported by the Thornton Endowment in Animal Science at Texas Tech University.

Effects of starch and rumen-protected histidine in a low-protein diet on production and nitrogen efficiency of dairy cows

R. Peng[1], A. Schudel[1], M. Niu[1], S.E. Räisänen[2]
[1]ETH Zurich, Zurich, Switzerland. [2]Aarhus University, Tjele, Denmark (susanna.raisanen@anivet.au.dk)

This study aimed to investigate interactions between dietary starch level and rumen-protected His (RPHis) in lactating cows fed MP-deficient diets (85% of requirements) on nutrient utilization, milk production, and plasma AA concentrations. Twelve multiparous Holstein cows [(136 ± 53.1 days-in-milk, 36 ± 3.0 kg/d milk yield (MY)] were assigned to a replicated 4×4 Latin Square design with a 2 x 2 factorial arrangement: 1) high starch (HS; 14% CP, 26% starch, 38% NDF), 2) HSRPHis (HS + RPHis), 3) low starch (LS; 14% CP, 16% starch, 44% NDF); 4) LSRPHis (LS + RPHis). The RPHis (62% His DM-basis, 84% estimated bioavailability) was supplemented to meet NASEM (2021) targeted digestible His supply (59 g/d) in LSRPHis and HSRPHis diets, and the digestible His-to-NE_L ratio (1.6 g/Mcal; Räisänen et al., 2023) in HSRPHis. Diets were balanced with RPHis coating and rumen-protected Lys and Met. Data were analyzed using linear mixed models in R. There were starch×RPHis interactions for MY ($P = 0.03$), lactose yield, and fat content ($P < 0.01$), but not for energy-corrected milk (ECM) yield, nutrient intake or utilization. Regardless of RPHis, HS diets tended to increase ($P = 0.06$) DMI and increased ($P = 0.03$) OM intake by 2%, but reduced CP and NDF intakes by 5% and 10.5%, respectively, with digestibility reductions of 10% and 16% ($P < 0.01$), likely due to starch-induced ruminal pH suppression. High-starch diets increased ($P < 0.01$) MY, ECM (+2 kg/d), and milk component yields, improving feed efficiency (+3%; $P = 0.02$), which were likely linked to elevated glucose availability. High-starch diets also enhanced N use efficiency (NUE, +17%), and reduced urinary N excretion (−29%), while fecal N excretion increased (+10%; $P < 0.05$). Irrespective of starch level, RPHis tended to reduce CP digestibility (−5%; $P = 0.07$) and tended to increase fecal N excretion ($P = 0.08$) without affecting milk N or NUE. Conversely, RPHis reduced MY (−0.8 kg/d) and lactose yield in LS diet ($P < 0.05$). Milk fat content increased with RPHis in LS diet (+3%) but declined in HS diet ($P < 0.05$). Plasma total EAA, His and Leu concentrations increased with HS ($P < 0.05$), whereas His concentration tended to increase with RPHis (50 vs. 44 µ*M*; $P = 0.06$). Further, 3-methyl-His, Val, and Ile concentrations decreased with RPHis ($P < 0.05$). While starch enhanced ruminal N capture and glucose-driven milk synthesis, RPHis did not improve lactational performance. This may stem from overestimated RPHis bioavailability, labile His pools mobilization, and low supply of other EAA, in this short-term experiment. Results emphasize the role of starch in optimizing NUE and production in low-protein diets, whereas responses to RPHis require further validation and considerations of overall AA supply and optimization under sustained MP deficiency.

References

NASEM (National Academies of Sciences, Engineering, and Medicine). 2021. Nutrient Requirements of Dairy Cattle. 8th rev. ed. The National Academies Press. https://doi.org/10.17226/25806.
Räisänen, S.E., Lapierre, H., Price, W.J., Hristov, A.N., 2023. Journal of Dairy Science. https://doi.org/10.3168/jds.2022-22966

The effect of protein to energy balance on performance in Holstein calves

K.A.M. Winters, M. van de Vosse, L.C.M. van Enckevort
Denkavit, Voorthuizen, Netherlands (k.winters@denkavit.nl)

Protein and energy are both essential for growth, however, the optimal ratio for balanced body development of young calves is unknown. Considering a 40 kg healthy Holstein calf in thermoneutral conditions, metabolisable energy (ME) and crude protein (CP) requirements are estimated at 4.4-5.5 Mcal/d and 238.7-264.9 g/d in the first to fifth weeks of life for 800 g/d weight gain, respectively (NASEM, 2021). This results in an estimated CP:ME ratio between 48.2 – 54.3 g CP/Mcal ME. This study aims to evaluate the effect of different CP:ME ratio on performance of calves. Treatments were iso-energetic milk replacers (MR; 4750 Kcal/kg) with different CP:ME: A (43.6 g CP/Mcal ME), B (47.4 g CP/Mcal ME), C (50.6 g CP/Mcal ME) and D (55.4 g CP/Mcal ME). Four hundred male calves (46.0±4.2kg, 17±3 days old, n = 100) were enrolled and placed into pens of 5 calves as part of a complete randomized block design with 4 blocks in 2 departments with 5 pens per row. The trial lasted 12 weeks and during 9 weeks MR (mixed at 150 g/L) was fed twice daily at 3 L with a 4-week step down period (6, 5, 4, 3, 2 L/d). From week 10 onwards, calves were completely weaned. In total all calves received 42 kg MR/calf. Solid feed intake (concentrate + chopped straw) was fixed between treatments to exclude the effect of differences in nutrient intake from solid feed on growth. Body weight was measured at arrival and 2, 6, 10 and 12 weeks thereafter. The data were analyzed using ANOVA followed by Tukey's HSD multiple comparison test to identify significant differences between groups. Data is presented as grouped averages ± standard error of the mean. In the first six weeks, average daily gain (ADG) was greater in group D compared to group A (791.8 g/d ± 17.92 vs. 726.0 g/d ± 16.61; P_tukey = 0.037). Post-weaning (weeks 10 – 12) calves fed with MR C (1527.6 g/d ± 27.05) grew faster than A (1407.9 ± 25.45; P_tukey = 0.004) and D (1421.9 g/d ± 33.64; P_tukey = 0.023). From start till the end at 12 weeks, no significant differences were observed in the ADG of the groups (P_anova = 0.446). Calves fed with greater CP:ME MRs in this trial had improved performance in the first six weeks which was not maintained during weaning, resulting in non-significant differences in body weight at week 12.

Reference

NASEM (National Academies of Sciences, Engineering, and Medicine). 2021. Nutrient Requirements of Dairy Cattle. 8th rev. ed. The National Academies Press. https://doi.org/10.17226/25806.

Evaluating metabolic and fermentation heat loss in dairy cows under negative energy balance using exhalomics to differentiate breath from ruminal exhaled gases

M.A. Barrientos-Blanco, U. Arshad, M. Niu
ETH Zürich, Zürich, Switzerland (mario.barrientosblanco@usys.ethz.ch)

Physiological models of energy partitioning assumes that fermentation heat loss (FHL) equals urinary energy. Our study aimed to explore a method to quantify the FHL in dairy cows. Twelve multiparous Holstein cows (100 ± 20 days in milk; 43.4 ± 6.77 kg/d milk yield) were enrolled in a crossover design (2 week of adaptation and 1 week of caloric restriction) and randomly assigned to 2 treatments: 1) control (CON) or negative energy balance (NEB). The CON and NEB diets contained 17.5 vs. 17.2 MJ/kg of DM of gross energy (GE), 38.7 vs. 50.7% NDF, 15.9 vs. 10.5% CP, and 16.5 vs. 7.9% starch, respectively. Milk yield and quality, and DMI were monitored daily. The gases (CO_2, O_2, and CH_4) were measured using GreenFeed, paired with spot fecal and urine sampling. The sampling for gases, urine and fecal was conducted 8 times during the last 3 days of each period, to represent every 3 hours of the day. The GE and nitrogen content of diet, fecal, urine, and milk were determined through calorimetric bomb and Dumas method, respectively. Levels of non-esterified fatty acids (NEFA) were analyzed from blood collected at 0700 h on days 1 - 4 and 7 of caloric restriction period. Implementing an established method (Barrientos-Blanco., 2025), we differentiated exhalome (Ex, ruminal eructation + breath) and breath (Br) using a threshold of 150 mV CH_4, to get CO_2 and CH_4 emissions, and O_2 consumption for Ex and Br. The adjusted values of gases representing FHL were determined as: FHL = Ex – Br g/second. Heat production (HP) for Br (HP_{Br}) and FHL (HP_{FHL}) were calculated according to Brouwer (1965). Energy retention (ER) was determined as: ER = Diet – Urine – Fecal – Milk – CH_4 – HP_{FHL} – HP_{Br}. Data were analyzed using mixed model with fixed effects of diet, period, time and their interactions, and random effect of cow. Inducing caloric restriction reduced the mean DMI (27.0 vs. 21.7 ± 0.91 Kg of DM; $P < 0.01$) and GE intake (368 vs. 545 ± 34.8 MJ/kg of DM; $P < 0.01$) in NEB compared to CON. An interaction between diet and time was observed for NEFA ($P < 0.01$), as the concentrations increased from day 1 to 4 (0.38 from 0.23 vs. 0.11 from 0.09 ± 0.04 mmol/L) in NEB compared to CON, respectively. The caloric restriction also increased ($P < 0.01$) partitioning of energy towards urine (7.3 vs. 4.6%), feces (32.8 vs. 24.9%), CH_4 (6.8 vs. 5.2%), and HP_{Br} (34.1 vs. 27.2%) in NEB compared to CON. Nevertheless, the energy partitioned towards milk (22.2 vs. 21.5%) or HP_{FHL} (2.3 vs. 2.0%) did not differ across treatments. These findings demonstrate that the major heat energy loss likely originates from metabolic activity rather than fermentative energy production.

References

Brouwer, E. 1965. Report of sub-committee on constants and factors. in Proc. Energy Metabolism. Proc. 3rd Symp., 1965. Academic Press.

Barrientos-Blanco, M.A., Arshad, U., Giannoukos, S., Islam, M.Z., Kunz, C., Peng, R., Räisänen, S.E., Zenobi, R., Niu, M., 2025. JDS Communications. https://doi.org/10.3168/jdsc.2024-0732

Funding

This study was funded by the Swiss National Science Foundation (Bern, Switzerland; grant no. 10001647).

Impact of flint corn processing method and urea source on nitrogen metabolism and its utilization efficiency in Nellore heifers fed high-concentrate diets

R.A. Lino[1], G.P. Silva[1], I.G. Fávero[1], J.M.C. Souza[1], L.K. Huang[1], I.P.C. de Carvalho[2], J. Martín-Tereso[2], E.D. Batista[1]
[1]Universidade Federal de Lavras, Lavras, Brazil. [2]Trouw Nutrition R&D, Boxmeer, Netherlands (erick.batista@ufla.br)

The low efficiency of nitrogen (N) utilization (ENU) in ruminants affects both metabolism and the environment, as unutilized N is primarily excreted through feces and urine. Urinary urea-N is the main form of N excretion, significantly contributing to environmental losses. Incorporating feed-grade urea (U) as a non-protein nitrogen source can reduce feeding costs and greenhouse gas emissions associated with land use. However, its rapid ruminal solubilization causes ammonia peaks that may exceed microbial assimilation capacity, increasing N losses. On the other hand, post-ruminal urea infusion can prolong N availability and enhance ruminal ENU (Oliveira et al., 2020). In this context, optimizing N supply and fermentable energy availability from corn processing methods (CPM) may further improve microbial protein synthesis (MPS) and its utilization, as well as enhance ENU for N retention. This study aimed to evaluate the interaction effects of flint CPM [dry-ground corn (DGC) and rehydrated, ensiled corn (REC)] and urea sources [US; U and coated urea (CU)] on ruminal and whole-body N utilization in heifers fed high-concentrate diets (72% dry-matter basis). Eight rumen-cannulated Nellore heifers (321 ± 15.2 kg body weight) were assigned to a replicated 4 × 4 Latin square design, balanced for residual effects. Treatments followed a 2 × 2 factorial arrangement, combining two CPM with two US. The CU product was designed to partially shift urea release to the post-ruminal gastrointestinal tract (Rauch et al., 2023). The experiment lasted 104 days, divided into four 26-day periods. Each period included 14 days of diet adaptation, followed by N intake estimation (days 15–20) and total feces and urine collection (days 16–20). The MPS was estimated from urinary purine derivatives excretion (allantoin and uric acid). A tendency for CPM × US interaction was observed only for MPS ($P = 0.07$), with higher values in heifers fed REC+CU compared to other treatments. No differences ($P > 0.25$) were detected in N intake (135 g/d), fecal N excretion (39 g/d), urinary N (52 g/d) and urea-N (39 g/d) excretion, or N retention (43 g/d). Compared to heifers fed U-diets, those fed CU-diets exhibited greater MPS efficiency relative to N intake ($P = 0.02$; 785 vs. 690 g/kg). Trends were observed for MPS efficiency relative to ruminal digestible organic matter ($P = 0.07$; 158 vs. 146 g/kg) and whole-body ENU ($P = 0.09$; 47 vs. 44% of digested N). Feeding REC-diets improved ENU for MPS ($P = 0.01$; 798 vs. 678 g/kg). Despite the lack of significant differences in N intake and excretion, CU inclusion in high-concentrate diets positively influenced N utilization for MPS and N retention. Additionally, feeding REC-diets improved ENU for MPS.

References

Oliveira, C.V.R., Silva, T.E., Batista, E.D., Rennó, L.N., Silva, F.F., de Carvalho, I.P.C., Martín-Tereso, J., Detmann, E., 2020. British Journal of Nutrition 124, 1166-1178. https://doi.org/10.1017/S0007114520002251

Rauch, R., Nichols, K., de Carvalho, I.P.C., Daniel, J.B., Martín-Tereso, J., Dijkstra, J., 2023. Journal of Animal Physiology and Animal Nutrition 109, 64-75. https://doi.org/10.1111/jpn.14034

Funding

This study was funded by Trouw Nutrition (Boxmeer, the Netherlands). The authors would like to thank FAPEMIG, CNPq, and NEPEC for their support.

Relationships among body weight changes, milk production and blood free fatty acid and ketone concentrations in dairy cows

T. Kokkonen[1], M. Hovinen[2]
[1]Department of Agricultural Sciences, Faculty of Agriculture and Forestry, University of Helsinki, Helsinki, Finland. [2]Department of Production Animal Medicine, Faculty of Veterinary Medicine, University of Helsinki, Helsinki, Finland (tuomo.kokkonen@helsinki.fi)

The amount of lipid reserves and body weight (BW) of dairy cows changes greatly during lactation and fluctuates over subsequent lactations. High body fatness at the time of calving is a risk factor for metabolic diseases. Further, excessive gain and concomitant mobilization of lipid reserves during successive lactations can be inefficient use of feed energy. The objective of the current study was to evaluate differences in milk production and blood free fatty acid and ketone concentrations based on body weight change categories. The data was composed of daily observations of milk yield and BW recorded with automatic milking system (AMS). Milk composition was analyzed monthly, and blood samples were collected from a subset of cows during lactation weeks 2 and 3. Criteria for cows to be included in the data were milk production and BW recordings from at least two subsequent lactations. Changes of BW were calculated between lactation days 7, 91 and 287. In total, 127 Nordic red cows and 344 lactations from parities 1 to 9 were entered to the data. Cows had free access to partial mixed ration. Changes of BW at each interval were divided to quartiles. Differences among quartiles in milk production performance and blood composition were analyzed with variance analysis. Statistical model included BW change quartile and parity as fixed factors. The cows in the highest quartile of BW gain (on average +0.5 kg/d) between lactation days 91 and 287 had 19% lower energy corrected milk (ECM) 305 d yield (9447 vs. 11617 kg) compared to the lowest quartile (+0.08 kg/d). When the quartiles were based on BW gain between lactation days 91 and 287 in previous lactation, the corresponding difference in following lactation ECM was 9% between cows with lowest and highest BW gain. During early lactation (between days 7 and 91), the cows in the lowest BW loss quartile (+0.40 kg/d) had 9% lower ECM compared to the highest yielding cows in the second highest quartile of BW loss (-0.08 kg/d). There was a moderate positive correlation between BW gains at two subsequent lactations during days 91 and 287 (r = 0.47). In contrast, the correlation between BW losses in two adjacent lactations was weak (r = 0.27) and no significant correlation was observed between BW gain in previous lactation and BW loss in following lactation. Cows in the highest BW loss quartiles had the highest plasma NEFA and BHB concentrations. There was a weak positive correlation (r = 0.27) between NEFA concentrations in the adjacent lactations. In conclusion, the relationships between BW gains and ECM yields of subsequent lactations suggest genetically driven differences in the efficiency to partition feed energy to milk.

Funding

Project received funding from The Finnish Association of Academic Agronomists

Mitochondrial protein acetylation in liver biopsies of pasture-based dairy cows

M. García-Roche[1,2], M. Carriquiry[1], A. Cassina[2], C. Quijano[2]
[1]Departamento de Producción Animal y Pasturas, Facultad de Agronomía, Universidad de la República, Montevideo, Uruguay. [2]CEINBIO, Facultad de Medicina, Universidad de la República, Montevideo, Uruguay (garciaroche.m@gmail.com)

Hepatic mitochondrial function is impaired in high-yielding dairy cows in pasture-based systems and acetylation may be a plausible inhibitory mechanism (García-Roche et al., 2023). This study explores protein levels of acetylated mitochondrial enzymes relevant in energy metabolism and antioxidant defenses and sirtuins (deacetylases) in high-yielding dairy cows during lactation in two pasture-based systems. Multiparous Holstein cows (n = 16, 563 ± 29 kgBW, 3.1 ± 0.1 units BCS, autumn calving) were assigned at calving to two feeding strategies (FS) that differed in the amount of grazed pasture while levels of energy-protein concentrate were similar. Grazed pasture represented 33% of estimated dry matter intake (DMI) in the fixed pasture (FixP) treatment at 21 and 180 DPP and varied from 41 to 51% of estimated DMI in the maximum pasture (MaxP) treatment from 21 to 180 DPP. Liver biopsies were collected at 21 and 180 DPP and western blots were performed against acetylated lysine (AcK), manganese superoxide dismutase (MnSOD), MnSOD acetylated lysine 68, ATP synthase subunit O (ATP5O), ATP5O acetylated lysine 139, sirtuins (SIRT) 3 and 5 and beta-actin in enriched mitochondrial fractions. Hepatic gene expression of transcription factors and coactivators: *SIRT1*, peroxisome proliferator-activated receptor alpha (*PPARA*), peroxisome proliferator-activated receptor gamma coactivator 1-alpha (*PGC1A*) and *SIRT3* were studied using real-time PCR. Data were analyzed using a mixed model that included DPP, feeding strategy (FS) and their interaction as fixed effects. Energy corrected milk was greater at 21 *vs.* 180 DPP and greater for MaxP than FixP (38.5 *vs.* 35.7 ± 1.3 for 21 DPP and 28.3 *vs*. 25.5 ± 1.3 for 180 DPP for MaxP and FixP, respectively, $P < 0.05$). Hepatic mitochondrial AcK levels were affected by FS and greater for MaxP (1.95 *vs.* 1.30 ± 0.43, $P < 0.01$). The ratios between acetylated and total MnSOD and acetylated and total ATP5O were affected by DPP and greater at 21 than 180 DPP (0.92 *vs.* 0.75 ± 0.06, $P < 0.05$ for MnSOD and 1.06 *vs*. 0.55 ± 0.14, $P < 0.01$ for ATP5O). In addition, the ratio between acetylated and total MnSOD was 1.3-fold greater in MaxP than FixP ($P < 0.05$). An interaction between DPP and FS was observed for levels of SIRT3 as they were greater for MaxP at 180 DPP ($P < 0.01$), no differences were observed in levels of SIRT5. No differences were observed in gene expression of *PGC1A*, *PPARA*, *SIRT1* or *SIRT3* underlining the importance of studying post-translation regulation of key enzymes. Acetylated MnSOD is activated by SIRT3 enhancing reactive oxygen species scavenging activity, a key mechanism in fatty liver protection (Nassir, 2020). In sum, the greater levels of SIRT3 observed in MaxP cows during mid-lactation suggest the activation of potential mechanisms to counteract hepatic mitochondrial acetylation, evidencing dysregulation of this mechanism during early lactation.

References

García-Roche, M., Quijano, C., Cassina, A., Peralta, R.A., Carriquiry, M., 2023. PLoS One 18, e0290551. https://doi.org/10.1371/journal.pone.0290551
Nassir, F., 2020. Exploratory Research and Hypothesis in Medicine 1, 248–258. https://doi.org/10.37349/emed.2020.00017

Funding

The project was funded by Comisión Sectorial de Investigación Científica, Universidad de la República I+D 2018-103 (M. Carriquiry, C. Quijano); Agencia Nacional de Investigación e Innovación FSA_1_2018_1_152220 (M. Carriquiry, A. Cassina) and Programa de Desarrollo de las Ciencias Básicas-Programa Despegue Científico 2023/2024 (M. García-Roche).

Effects of protein supplementation, source, and processing on digestibility and gas exchange in Jersey steers fed a hay-based diet

P. Pucetti, C. Finck, J.T. Silva, Z. Carlson, K.C. Swanson
North Dakota State University, Fargo, USA (pauliane.pucetti@ndsu.edu)

High-fiber diets with low protein content, often used during drought or winter, can impair microbial activity and reduce energy and nitrogen utilization in cattle. Strategic protein supplementation can improve fiber digestibility and nutrient absorption, potentially enhancing metabolic efficiency and reducing environmental impact. This study evaluated the effects of supplementing dried distillers' grains with solubles (DDGS) or soybean meal (SB), processed as meal or pelleted, on dry matter (DM) and neutral detergent fiber (NDF) intake and digestibility, methane (CH_4) and carbon dioxide (CO_2) production, oxygen consumption (O_2), and respiratory quotient (RQ) in Jersey steers fed a grass hay-based diet. Five rumen-cannulated Jersey steers (BW: 424 ± 45 kg) were used in a 5 × 5 Latin square design. Each period consisted of 7 days of diet adaptation, 5 days of total fecal collection, and one day (24 h) of gas exchange measurements using open-circuit respiration head boxes. Diets consisted of grass hay (8.66% crude protein, CP) offered ad libitum, with no supplementation (control), or with protein supplements provided either as meal (DDGS: 32.46% CP; SB: 27.68% CP) or pelleted (DDGSP and SBP) at 0.5% of body weight (DM basis). Gas outputs were expressed as L/day, and per kg of DM and NDF digested. Data were analyzed using a mixed model in SAS (PROC MIXED), with treatment as a fixed effect and steer and period as random effects. Pre-planned contrasts assessed supplementation, source, processing, and their interaction. Effects were considered significant at $P \leq 0.05$ and trends at $0.05<P\leq 0.10$. Treatment had no effect on DM intake ($P>0.10$), while NDF intake tended to be higher with DDGS than SB ($P=0.069$). DM digestibility increased with protein supplementation ($P<0.001$) and was greater with pelleted supplements ($P=0.010$). NDF digestibility was greater with DDGS ($P=0.006$) and with pelleting ($P=0.004$). O_2 consumption tended to increase with supplementation in absolute terms ($P=0.096$) and increased per digested NDF ($P=0.008$). Pelleting reduced O_2 consumption per digested NDF ($P=0.042$) and tended to reduce O_2 consumption per digested DM ($P=0.064$). CO_2 production tended to increase with supplementation per digested NDF ($P=0.057$), while pelleting reduced CO_2 per digested NDF ($P=0.039$) and tended to reduce CO_2 per digested DM ($P=0.061$). RQ decreased with pelleted supplements ($P=0.048$). CH_4 production was not affected in absolute terms but was reduced with supplementation when expressed per digested DM ($P=0.016$). Supplement source and the interaction did not influence any of the gas exchange variables. Protein supplementation improved DM digestibility and reduced CH_4 per digested DM. Pelleting enhanced fiber digestibility, reduced O_2 and CO_2 per digested NDF, and lowered RQ, suggesting changes in fermentation or metabolic efficiency.

Funding
This research was supported by the North Dakota Soybean Council.

A moderate nutrient restriction before dry-off decreases milk yield without severely impairing metabolism

L. Cattaneo, F. Piccioli-Cappelli, A. Minuti, E. Trevisi
Department of Animal Science, Food and Nutrition (DIANA), Faculty of Agricultural, Food and Environmental Sciences, Università Cattolica del Sacro Cuore, Piacenza, Italy (luca.cattaneo@unicatt.it)

Drying-off is a stressful period for dairy cows, especially when milking ceases abruptly while milk production remains high. A safety threshold of 15 kg/d at dry-off has been suggested, with various strategies employed to reach this level while supporting mammary involution, particularly in the context of selective dry cow therapy. Providing only grass hay before dry-off successfully lowered milk yield but significantly impacted metabolism during this phase (Cattaneo et al., 2024). Therefore, the present study aimed at assessing whether a moderate nutrient restriction at drying-off would reduce yield without severely affecting metabolism. Twenty-two Holstein cows with yield greater than 15 kg/d were fed either the dry period diet for 5 d before dry-off (DryD, n = 11, 12.5% CP and 13.0% starch) or continued to receive the lactation diet until milking cessation (LacD; n = 11, 16.5% CP and 26.5% starch). Both groups were dried off with a single infusion of antibiotics and internal teat sealant after last milking. After dry-off, both groups received the same dry diet until calving. Milk yield and feed intake were continuously monitored. Milk samples were collected before dry-off and after calving to determine composition and SCC. Blood samples were collected at –7, 0, 3, and 7 days relative to dry-off (DRD) and at –7, 7, and 28 days relative to calving (DRC) to assess blood cell count and plasma metabolic profile. Rumen fluid samples were collected at –7 and 0 DRD to measure volatile fatty acids. Data were analyzed with repeated measures mixed models using data collected before restriction as a baseline. DryD significantly reduced milk yield at dry-off (–7.5 kg/d) and DMI (–9 kg/d) compared with LacD. After drying, DMI was similar between groups. BCS slightly decreased in DryD at dry-off, while rectal temperature did not differ between treatments. Milk fat and SCC slightly increased at dry-off with DryD, although not significantly. In rumen fluid, DryD tended to increase pH and decreased ammonia, lactate and total VFA concentrations. The molar proportions of acetate, isobutyrate, and isovalerate increased, whereas those of propionate, butyrate, and valerate decreased. Plasma concentrations of total protein, globulin, Zn were reduced in DryD after dry-off, and urea, ceruloplasmin, and reactive oxygen metabolites tended to be reduced. Other markers investigated were unaltered, including haptoglobin, NEFA, and BHB. Overall, feeding the dry period diet before dry-off decreased milk yield and seemed to improve the response to dry-off, particularly from an inflammatory standpoint. The impact on milk production and metabolism was milder compared with the severe nutrient restriction strategy previously tested. The impact on the next lactation will be evaluated. Future efforts should evaluate whether the obtained yield reduction was sufficient with the aim of selective therapy at dry-off.

Reference

Cattaneo, L., Lopreiato, V., Piccioli-Cappelli, F., Dahl, G.E., Trevisi, E., Minuti, A., 2024. Journal of Dairy Science 107, 5090-5103. https://doi.org/10.3168/jds.2023-24017

Funding

E.T. and A.M. are supported by the project CRUMLIS, co-financed by the Italian Ministry of Foreign Affairs and International Cooperation. L.C. is supported by the project NODES which has received funding from the MUR – M4C2 1.5 of PNRR funded by the European Union – NextGenerationEU (Grant agreement no. ECS00000036).

Safety of increasing levels of a urea-biuret mixture on beef cattle health

A. Maderal[1], I. Fernandez-Marenchino[1], F. Tarnonsky[1], A. Lacherre[1], C. Gomez-Lopez[1], F. Podversich[1], A. Fernandez-Lehmann[1], M. Larrauri[1], W. Cuervo[1], T. Andres-Soto[1], G. Dubeux[1], J. Infante-Cangrejo[1], M. Venturini[1], D. Heredia[1], A. Gonella-Diaza[1], T.M. Schulmeister[1], I. Ruiz-Ascacibar[2], I. Ipharraguerre[3], N. DiLorenzo[1]

[1]North Florida Research and Education Center, University of Florida, Marianna, USA. [2]Yara Industrial Solutions, Madrid, Spain. [3]Institute of Human Nutrition and Food Science, University of Kiel, Kiel, Germany (ndilorenzo@ufl.edu)

Non-protein nitrogen (NPN) has been widely used in cattle nutrition as a cost-effective solution to improve animal performance, especially in low-protein diets. Urea is the primary source of NPN used for cattle supplementation with a recommended limit typically up to 1% of the diet due to potential toxicity risks; however, NPN sources that combine urea and biuret (UB) may exhibit slower degradation rates. Thus, we hypothesize that inclusion of NPN in beef cattle diets can be safely increased by using a UB mixture, thereby avoiding potential risks. The objective of this study was to assess the effects of supplementing 1% urea and incremental UB mixture inclusions on cattle performance, health, and tissue residues. To this aim, 128 Angus-crossbred steers were used in a generalized randomized block design. Treatments included a corn-silage basal diet supplemented with 1% urea (U1) or UB mixture at 1.12% (UB1), 2.24% (UB2), or 4.48% (UB4) of the diet dry matter. The experimental period consisted of 24 d of adaptation to NPN sources, followed by 86 d of performance data collection. Blood samples from 64 steers were collected before NPN sources were supplemented (d 21) and at the end of the performance period (d 86). Then, a subset of 16 animals were euthanized for organ and tissue examination and for cyanuric acid and ammelide residues analysis. No differences were observed across treatments for dry matter intake ($P >$ 0.05); however, the gain to feed ratio (G:F) decreased in UB4 compared with U1, though no differences in G:F were observed between U1, UB1 and UB2. Blood urea nitrogen (BUN) concentration increased ($P <$ 0.001) as the UB inclusion increased (UB2 and UB4). Total protein (TP) and albumin were not affected ($P >$ 0.05) by treatment ($P >$ 0.05). Liver enzymes, such as aspartate aminotransferase, alanine aminotransferase, and gamma-glutamyl transferase, remained stable ($P >$ 0.05) and within the bovine reference range. Hematology parameters, packed cell volume and hemoglobin, increased ($P <$ 0.05) in UB4 compared with U1 but remained within the normal reference range. Necropsy examinations revealed no gross pathological abnormalities. Residues of cyanuric acid were not detected in any tissue, and UB4 showed only trace amounts of ammelide (< 2 mg/kg) in liver tissue. While UB2 and UB4 may exceed the optimal NPN level, only UB4 led to a reduction in efficiency. As expected, BUN levels simultaneously increased with increasing inclusion of UB; however, TP remained constant, which indicates that liver function, such as protein synthesis, was not impaired despite the higher NPN intake. The combination of UB mixtures evaluated in this study demonstrate the safety and efficacy of increasing inclusion of NPN sources in cattle diets beyond the typical 1% limit for urea, without compromising animal health.

Funding

This work was supported by Yara International ASA.

Milk performance of Holstein cows in response to decreased concentrate allowance from an out-of-parlour feeder

J.B. Daniel, J. Martín-Tereso
Trouw Nutrition R&D, Amersfoort, Netherlands (Jean-Baptiste.Daniel@trouwnutrition.com)

Ad libitum partial mixed ration feeding with additional concentrate offered at milking or in an out-of-parlour feeder is a common strategy, allowing individual concentrate allocation based on milk production, parity, and lactation stage. In this study, we evaluated the effect of decreasing the allowance of two different concentrates from a reference concentrate feeding scheme (REF). This reference scheme provided a fixed amount of 2.3 kg of a high protein concentrate (HP; 29.8% protein and 17.5% starch) to all cows, and a variable amount of a high starch concentrate (HS; 19.4% protein and 27.4% starch) based on parity and level of milk production (from 0 to 8.5 kg/day). A total of 60 lactating Holstein cows (28 were primiparous; 173 ± 78 days in milk) were used in a randomized complete block design, and assigned to one of the four dietary treatments for 8 weeks: REF, REF with 1 kg less of HP (-HP), REF with 1 kg less of HS (-HS), and REF with 1 kg less of HP and 1 kg less of HS (-HPHS). For analysis (Proc Mixed, SAS), the fixed effects of treatment, week, and their interaction were included, along with a covariate (the average from the last week before the study began). The effect of block was also included as a random effect. Cows fed -HP and -HPHS consumed 1.2 kg less HP ($P < 0.001$), and cows fed -HS and -HPHS consumed 1.1 kg less HS ($P < 0.001$), as compared to REF cows (total concentrate intake of 6.8 kg DM). The substitution rate (Δ kg PMR per Δ kg concentrate, relative to REF) was 0.38, 0.17 and 0.41 for -HP, -HS, and -HPHS, respectively. Compared to REF (23.6 kg/d), dry matter intake was lower for -HP, -HS, and -HPHS (22.6, 22.5, and 21.9 kg/d, respectively), with reductions being significant ($P < 0.05$) except for -HP ($P = 0.08$). Milk fat yield and content were not significantly affected by treatments ($P = 0.19$ and 0.13, respectively). Milk protein yield was reduced by approximately 88 g/d for -HP and -HPHS compared to REF and -HS ($P < 0.05$). However, only -HPHS had a significantly lower milk protein content than REF ($P < 0.05$). Marginal milk protein yield response (Δ kg milk protein yield per Δ kg protein intake, relative to REF) was 0.30, 0.07 and 0.17 for -HP, -HS, and -HPHS, respectively. Milk N efficiency was increased for -HPHS as compared to REF (34.3 vs. 32.7 %, $P < 0.05$). Finally, cows fed -HPHS had a lower body weight than those on other treatments (644 vs. 650-653 kg, $P <0.05$). Overall, reducing the allowance of HP had more pronounced impacts on milk productivity than reducing HS.

Funding

The study was financed by Trouw Nutrition R&D (Amersfoort, the Netherlands).

Quantifying individual variation in milk true protein production in response to varying levels of metabolizable protein and amino acid supply in dairy cows

L.M. Campos[1,2], K. Frady[3], K. Kim[1], J. Saunders[1], I.A.M.A. Teixeira[4], A. Rius[3], M.D. Hanigan[1]
[1]Virginia Tech, Blacksburg, USA. [2]AgNext -- Colorado State University, Fort Collins, USA. [3]University of Tennessee, Knoxville, USA. [4]University of Idaho, Twin Falls, USA (mhanigan@vt.edu)

This study aimed to quantify individual milk protein production (MTPP; kg/d) responses and variance across dairy cows fed different levels of dietary metabolizable protein (MP) and metabolized amino acids (AA). The experiment was conducted in 3 phases (P1, P2, P3) using 56 Holstein lactating cows. Dietary treatments were implemented using an automated feeding system (AFS). Diets were constructed to provide 80% of predicted dry matter intake (DMI) via a partial mixed ration and 20% via 2 concentrate blends (low and high MP) delivered by the AFS. All animals were fed to NASEM (2021) requirements in P1 to establish a baseline. In P2, animals were assigned to replicated 5x5 Latin squares and fed 5 diets with MP concentrations of 2.40, 2.52, 2.65, 2.78, 2.91 kg/d. In P3, animals were randomly split in two cohorts (28 cows each) and assigned into replicated 4x4 Latin squares fed a 2.61 kg/d of MP diet. Treatments in P3 were supplementation with 0, 48, 70, and 92 g/d metabolized Lysine; or 0, 26, 38, and 50 g/d of metabolized Histidine. Individual DMI was assessed based on a model informed by chewing activity, dietary predictors, and intake markers. The MTPP was regressed on individual MP or metabolized AA intake. Responses were classified as quadratic (QR), linear (LR), or non-responders (NR). In P2, 34% of the cows had a QR, averaging (variance) 1.35 kg/d (0.021 kg^2/d) of MTPP, while 52% of the cows had a LR, averaging 1.37 kg/d (0.017 kg^2/d). In P3, Lysine cohort outcomes indicated 33% of cows classified as QR, averaging 1.33 kg/d of MTPP, while 50% were LR with an average of 1.41 kg/d. Histidine cohort indicated that half of the cows were classified as QR averaging 1.44 kg/d, while 33% were LR with an average of 1.38 kg/d. According to NASEM (2021) and Hanigan et al. (2024), Lysine and Histidine are positive drivers of MTPP, evidenced by positive slope coefficients, with the best equations including both AAs as predictors. Hanigan et al. (2024) suggested that, except for Leucine, the relationship between milk true protein production response and MP supply within the in vivo range primarily exhibits a linear pattern, although there is evidence of some nonlinearity due to the presence of a quadratic term in the model. In summary our findings reveal response heterogeneity within a single pen and underscore the importance of accounting for individual animal variation in nutrient response to better align nutrient supply with cow-specific needs.

References

NASEM. 2021. National Academies of Sciences, Engineering, Medicine. Nutrient requirements of dairy cattle. Washington: National Academies Press.
Hanigan, M.D., Souza, V.C., Martineau, R., Lapierre, H., Feng, X., Daley, V.L., 2024. Journal of Dairy Science 107, 5587-5615. https://doi.org/10.3168/jds.2024-24230

Funding

The authors thank Balchem Corporation and Vetagro S.p.A. for their funding support.

Phase feeding high protein TMRs to fresh cows; concurrent and carryover effects on milk and milk component yields of multiparous Holstein Friesian cows

M.B. Samarasinghe, M.R. Weisbjerg, M. Larsen
Department of Animal and Veterinary Sciences, Aarhus University, Tjele, Denmark (bhagya.samarasinghe@anivet.au.dk)

The aim of the study was to investigate whether feeding a high protein TMR during first 7 d in milk or first 28 d in milk would result in increased concurrent and carryover milk yields thereby improving N utilization. Forty-eight multiparous Holstein Friesian cows were used in a randomized block design where cows continuously entered the treatments according to a predefined randomized order. Cows were blocked by parity and treatments of another trial carried out during far-off dry period. Three treatments started 3 ± 2 h after calving: 1) a standard lactation TMR having a normal protein content (Con-TMR; 16.3 % CP; n = 16), 2) TMR with a high protein content during first 28 d (i.e., 28.3 ± 1 d) after calving (28d-TMR; 21.3 % CP; n = 16), and 3) a very high protein TMR during first 7 d after calving (7d-TMR; 27 % CP; n = 16), followed by feeding all three groups with Con-TMR until 150 d in milk. The protein content of high protein TMRs was achieved by replacing sugar beet pulp with SoyPass and maize gluten meal. The statistical model included treatments, parity, day or week in milk as fixed effects, and cow, block and season as random effects. Preliminary results showed that DMI during the first 28 d in milk was affected by the treatments ($P = 0.02$) where the 28d-TMR group had a higher DMI compared to Con-TMR and 7d-TMR groups during the first 2 weeks in milk. The DMI did not differ among the treatments during the follow up period. Milk yield tended to show an interaction between treatment and day on 5 d to 14 d in milk ($P = 0.08$) and a significant effect from 14 d to 35 d in milk ($P < 0.01$). The average daily milk yield of 28d-TMR (44.4 ± 2.4 kg/d; $P = 0.1$) tended to be higher than the Con-TMR and 7d-TMR (40.6 ± 2.4 kg/d) in week 3 in milk. After week 4 in milk, milk yield did not differ among the treatments (follow-up period; $P = 0.74$). Milk protein yield was affected by the treatments during the first four weeks in milk ($P = 0.025$) where 28-d TMR and 7d-TMR had higher protein yields than Con-TMR. However, it was not affected during the follow-up period ($P = 0.73$). Milk fat and lactose yields were not affected by the high protein fresh cow TMR treatments. The increased CP content in fresh cow rations using SoyPass and maize gluten as rumen bypass protein sources led to increased milk and component yields during the feeding period, but these effects appeared not to persist in the follow up period.

Effect of feed restriction on the hepatic proteome of early lactation dairy cows in a pasture-based system

M. García-Roche, A. Astessiano, D. Custodio, G. Ortega, P. Chilibroste, M. Carriquiry
Departamento de Producción Animal y Pasturas, Facultad de Agronomía, Universidad de la República, Montevideo, Uruguay (garciaroche.m@gmail.com)

To study the effect of a five-day feed restriction on the hepatic proteome of early lactation dairy cows in a pasture-based system, six multiparous Holstein cows (477 ± 73 kg body weight (BW) and 2.75 ± 0.16 body condition score (BCS) units) were selected at 29 ± 10 days in milk (DIM). Cows underwent an adaptation period with *ad libitum* feeding (36.5 kgDM/cow daily pasture allowance and 5.3 kgDM/cow and day of an energy concentrate; from 29 to 38 DIM), they were later offered 50% of *ad libitum* diet (16.5 kgDM/cow daily pasture allowance and 2.6 kgDM/cow and day of an energy concentrate; feed restriction period from 38 to 43 DIM), and in the last period cows returned to the *ad libitum* diet (from 43 to 54 DIM; refeeding period). Milk yield was recorded daily, milk and blood samples were collected twice during every period and liver biopsies were collected at the end of every period. Plasma metabolites and hormones were measured with colorimetric and radioimmunoassay commercial kits, respectively. Liver homogenates were prepared and subjected to LC-MS/MS analysis. Milk yield and components, plasma metabolites and hormones were analyzed with a model that included period as fixed effect. Proteomic data was analyzed with Perseus software v1.6.1.3 and pathway enrichment analysis was performed with String software v.12.0. Energy corrected milk yield decreased during feed restriction (27.1 *vs.* 33.4 and 33.7 ± 1.7 kg/d in adaptation and refeeding periods, $P < 0.0001$). Plasma glucose levels tended to be lower during the restriction *vs.* the adaptation and refeeding periods (3.33 *vs.* 3.96 and 3.49 ± 0.24 mmol/L, $P < 0.1$) and plasma non-esterified fatty acids (0.58 *vs.* 0.40 and 0.33 ± 0.06 mmol/L, $P < 0.001$, NEFA) and beta-hydroxybutyrate (0.78 *vs.* 0.41 and 0.32 ± 0.16 mmol/L, $P < 0.01$, BHB) increased during feed restriction *vs.* the adaptation and refeeding periods, respectively. Plasma glucagon decreased during feed restriction (157 *vs*. 211 and 226 ± 30 pg/mL in adaptation and refeeding periods, $P < 0.01$) and insulin levels remained unchanged. A total of 1912 proteins were identified, with 16 differentially expressed (DEPs) between the adaptation *vs*. restriction period (fold change > 2 and p value < 0.05), 31 DEPs between the restriction *vs.* refeeding period and 19 DEPs between the adaptation *vs*. refeeding period. Pathway enrichment analysis revealed lipid biosynthetic processes, in particular cholesterol biosynthesis, were downregulated during feed restriction (FDR < 0.01). In sum, feed restriction led to lipid catabolism as observed from the higher levels of NEFA and BHB, opposing lipid biosynthesis and impairing steroid synthesis (Akbar *et al*., 2013). Impaired cholesterol synthesis could lead to decreased export of triglycerides and contribute to the development of fatty liver (Gross *et al.,* 2015).

References

Akbar, H., Bionaz, M., Carlson, D.B., Rodriguez-Zas, S.L., Everts, R.E., Lewin, H.A., Drackley, J.K., Loor, J.J., 2013. Journal of Dairy Science 96, 2201-2213. https://doi.org/10.3168/jds.2012-6036

Gross, J.J., Kessler, E.C., Albrecht, C., Bruckmaier, R.M., 2015. PLoS One 10, e0121956. https://doi.org/10.1371/journal.pone.0121956

Funding

The project was funded by Comisión Sectorial de Investigación Científica of Universidad de la República I+D 2018 ID 103 (M. Carriquiry and C. Quijano) and Dirección Nacional de Innovación, Ciencia y Tecnología, of Ministerio de Educación y Cultura FVF_2023_454 (M. García-Roche).

Comparison of milk oligosaccharides in Holstein-Friesian cows and water buffaloes during early lactation

L. Isernhagen, C.E. Galuska, A. Vernunft, A. Hoeflich, S.P. Galuska
Research Institute for Farm Animal Biology (FBN), Dummerstorf, Germany (galuska.sebastian@fbn-dummerstorf.de)

In addition to dairy cows, water buffaloes are an important livestock in the dairy industry with around 13% of the worldwide milk production (FAO, 2013), even though they have a lower milk yield than dairy cows (Faye and Konuspayeva, 2012). Besides important glycoproteins, such as lactoferrin and immunoglobulins, the milk of dairy cows as well as water buffaloes contains high levels of milk oligosaccharides (MOs), which additionally supports numerous beneficial processes in the offspring. For instance, MOs positively influence the maturation of the microbiome and immune system. Furthermore, MOs play a role in inhibiting the adhesion of various pathogens to epithelial cells to prevent invasion. To characterize in detail the structure and distribution of MOs during early lactation in Holstein-Friesian cows and water buffaloes, we used several analytical approaches like LC-MS to analyze the MOs from colostrum (day 0), transitional (first week of lactation) and mature milk (day 30). In both species, the majority of MOs were sialylated (cow: 29 of 37; buffalo: 55 of 88) and showed a rapid decrease during early lactation. Interestingly, only fucosyllactose was detectable in bovine milk, whereas 7 different fucosylated MOs were present in buffalo milk. In general, we observed a higher structural diversity of MOs in water buffalo. Because of the beneficial effects of fucosylated and sialylated MOs, their distribution could be an additional quality marker for the bioactivity of colostrum, transitional and mature milk.

References

FAO, 2013. Milk and dairy products in human nutrition (1st ed.). FAO. https://openknowledge.fao.org/handle/20.500.14283/i3396e
Faye, B., Konuspayeva, G., 2012. International Dairy Journal 24, 50-56. https://doi.org/10.1016/j.idairyj.2011.12.011

Interaction between dietary energy source and rumen protein degradability on nitrogen balance in dairy cows

M. Landry[1], P. Denis[1], F. Huot[1], C. Rheault[1], I.M. Rodriguez Lemus[2], Y. Lebeuf[1], D.E. Rico[3], P.Y. Chouinard[1], K. Nichols[4], R. Gervais[1]
[1]Université Laval, Québec, Canada. [2]Universidad de El Salvador, San Salvador, El Salvador. [3]Centre de recherche en sciences animales de Deschambault, Deschambault, Canada. [4]Department of Animal Science, University of California, Davis, USA (myriam.landry.8@ulaval.ca)

We investigated the interaction between dietary energy source and rumen protein degradability on N balance in dairy cows. Eight Holstein cows (95 ± 22 days in milk) were used in a replicated 4 × 4 Latin square design to test the effect of energy source (ES) and rumen undegradable protein (RUP) level using a 2 × 2 factorial arrangement of treatments. Treatments were isoenergetic diets (1.73 ± 0.03 Mcal/kg dry matter; DM) formulated to contain 21% starch and no added lipids (ST) or 11% starch (by replacing corn grain with soybean hulls) combined with 1.8% palmitic acid (FT; DM basis) and two levels of rumen undegradable protein [RUP; 4.5% (low) vs. 6.1% (high); DM basis], obtained by replacing non-treated with heat-treated soybean meal. Remaining starch was from corn silage. The ST and FT diets were isonitrogenous (15.1 ± 0.20% crude protein; DM basis). Each treatment period consisted of 14 days of adaptation and 7 days of data collection. The statistical model included cow as a random effect, and period, square, ES (ST or FT), level of RUP, and their interaction as fixed effects. Dry matter intake did not differ between treatments (28.8 ± 0.87 kg/d; $P \geq 0.22$). Nitrogen intake was not affected by ES ($P = 0.34$) and was smaller at the high RUP level (679 vs. 708 ± 20.9 g/d; $P = 0.03$). As a proportion of ingested N, fecal N excretion was not affected by ES ($P = 0.83$) and increased at the high RUP level (38.9 vs. 37.1 ± 0.82 %; $P = 0.02$). Urinary N excretion as a proportion of N intake was not affected by RUP level ($P = 0.55$) but increased in response to FT (23.5 vs. 21.0 ± 0.66 %; $P < 0.01$). Energy from FT decreased milk yield, but only when combined with low RUP (29.7 vs. 34.1 ± 1.92 kg/d; ES × RUP: $P < 0.01$). Milk N content decreased independently in response to high RUP and FT (0.511 vs. 0.524 ± 0.0230 %; $P < 0.01$). Milk N efficiency (milk N/N intake) was greater with ST than FT (25.5 vs. 23.2 ± 0.59 %), but when energy was provided from FT, milk N efficiency was smaller at the low RUP level (21.4 vs. 24.9 ± 0.59 %; ES × RUP: $P < 0.01$). Milk urea N tended to decrease at the high RUP level (10.9 vs. 11.4 ± 0.32 mg/dL; $P = 0.08$) and increased in response to FT (12.2 vs. 10.0 ± 0.32 mg/dL; $P < 0.01$). Milk N efficiency was greater with energy from ST than FT but could be improved when FT was combined with increased RUP.

Funding

This research was funded through the Quebec Consortium for Industrial Bioprocess Research and Innovation, Novalait, Innov'Action program of the Ministère de l'Agriculture, des Pêcheries et de l'Alimentation du Québec, and the National Sciences and Engineering Research Council of Canada.

Evaluating nitrogen use efficiency and predicting crude protein levels in dairy cattle diets: insights from a decade of nitrogen balance studies at ILVO

T. Van den Bossche, L. Vandaele, K. Goossens
ILVO, 9090 Merelbeke-Melle, Belgium (tine.vandenbossche@ilvo.vlaanderen.be)

Nitrogen (N) balance studies offer valuable insights into milk N efficiency (MNE) and N excretion in dairy cattle. Over the past decade (2015–2024), 13 such studies were conducted at the ILVO experimental research facility, aiming at evaluating the effects of feed additives and/or crude protein (CP) levels in the diet on MNE and N excretion. The data from these studies were combined into a dataset, which was used to develop models for predicting dietary CP content. These models could contribute to a monitoring system that helps farmers prove compliance with feeding strategies (low-protein diets) to reduce ammonia emissions. In these experiments, urine and feces samples were collected via total collection or pooled spot samples, with at least four samplings per pool. In case of spot samples, urinary volume was estimated using creatinine concentration, based on an average excretion rate of 29 mg per kg body weight per day (Tebbe & Weiss, 2018), and fecal production was estimated by the acid-insoluble ash content of feed and feces (McCarthy et al., 1974). The dataset contains data from 524 cow observations, except for blood urea (n = 336) as not all trials included blood sampling. Across all experiments (mean ± SEM), cows had a parity of 2.2 ± 0.1, were 171 ± 3 DIM, consumed 22.2 ± 0.2 kg of DM/day, and produced 33 ± 0.3 kg of milk/day. Dietary CP ranged between 11–19% (15.4 ± 0.1%), with mean DVE and OEB values of 92 g/kg DM and 185 g/day, respectively. From the total N intake (583 ± 5 g/day), 31.4% was allocated to milk N (183 ± 2 g/day) (MNE), 28.8% was excreted in urine (168 ± 2 g/day), and 34.8% was lost in feces (203 ± 2 g/day), with the remaining 5% attributed to body weight changes and metabolic losses. Urinary urea N (UUN) concentration was 4.66 ± 0.06 g/L, milk urea N (MUN) concentration was 101 ± 1 mg/L, and blood urea concentration was 21.0 ± 0.3 mg/dL. Various models were tested to predict the CP level of the diet, with the best model being: CP (%) = 10.70 + 0.13 × UUN (g/L) + 0.01 × milk N excretion (g/d) + 0.11 × blood urea (mg/dL). This model achieved the highest adjusted R^2 (0.61) and lowest AIC (785) and BIC (804) values. However, the second-best model, which excludes UUN, was: %CP = 10.98 + 0.009 × milk N excretion (g/d) + 0.13 × blood urea (mg/dL). This model yielded an R^2 of 0.60, AIC of 791, and BIC of 806. These findings highlight the potential of using milk, urine and/or blood parameters to predict the dietary CP level, with model 2 offering a more feasible option due to fewer sample requirements.

References

Tebbe, A.W., Weiss, W.P., 2018. Journal of Dairy Science 101, 5020-5032. https://doi.org/10.3168/jds.2017-14098

McCarthy, J.F., Aherne, F.X., Okai, D.B., 1974. Canadian Journal of Animal Science 54, 107-109. https://doi.org/10.4141/cjas74-016

Research progress on the effects of taurine on protein metabolism in beef cattle

G. Zhao, M. Li, S. Zhang, Y. Liu, J. Lan, J. Hu, M. Fan, B. Hou
China Agricultural University, Beijing, China (zhaogy@cau.edu.cn)

Taurine (Tau) is a non-proteinogenic amino acid not incorporated into body proteins. While animal-derived products contain Tau, it is absent in plant-based foods (Schaffer et al., 2014). In beef cattle, Tau is exclusively synthesized endogenously, primarily from methionine (Met) as the key precursor. However, this endogenous production is insufficient to meet physiological demands (O'Flaherty et al., 1997), and Tau deficiency may contribute to reduced nitrogen use efficiency (NUE). From 2022 to 2025, we conducted a series of experiments investigating Tau metabolism and its roles in beef cattle under the National Natural Science Foundation of China-funded project "Regulation and molecular mechanism of semi-essential amino acid taurine on body protein turnover in beef cattle" (Grant No. 32172748). Our findings revealed that supplementing unprotected Tau in the basal diet of beef cattle enhanced ruminal microbial crude protein (MCP) synthesis and fiber digestibility by modulating the rumen bacterial community, but it did not influence nitrogen retention (NR) or NUE. This result implied that Tau was highly hydrolyzable in rumen fermentation, and it was confirmed in a subsequent in vitro rumen fermentation experiment. To investigate the impact of Tau on the body protein metabolism in beef cattle, we supplemented rumen-protected Tau (RPT) to beef cattle and used ^{15}N-glycine as a tracer alongside with nitrogen balance trial. The results demonstrated that Tau effectively improved both body protein synthesis and protein degradation, thereby improving body protein turnover, NR and NUE. Further investigations with beef cattle indicated that Tau had similar impacts to Met on improving NR and NUE, and Tau improved ruminal MCP synthesis by upregulating rumen microbial pathways related to sulfur and amino acid metabolism. We also found that β-alanine supplementation suppressed Tau status but improved NR and NUE by altering plasma amino acid profiles and metabolome in beef cattle. In conclusion, Tau is highly hydrolysable in rumen fermentation. While unprotected Tau enhances ruminal MCP synthesis and fiber digestibility, its effects on NR and NUE are limited due to its high ruminal degradability. In contrast, supplementation with RPT improves body protein turnover, NR and NUE in beef cattle. These findings indicate that Tau deficiency is a key factor to low NUE in cattle. Furthermore, the results suggest that adequate Tau intake in humans may similarly improve body protein renewal and health.

References

O'Flaherty, L., Stapleton, P.P., Redmond, H.P., Bouchier-Hayes, D.J., 1997. European Journal of Clinical Investigation 27, 873-880. https://doi.org/10.1046/j.1365-2362.1997.2000747.x
Schaffer, S.W., Jong, C.J., Ito, T., Azuma, J., 2014. Amino Acids 46, 47-56. https://doi.org/10.1007/s00726-012-1414-8

Funding

Regulation and molecular mechanism of semi-essential amino acid taurine on body protein turnover in beef cattle (grant no. 32172748), supported by National Natural Science Foundation of China.

Indications of inflammation and cytokine activity in response to rapid fat deposition in beef steers

J. Thomson[1], N. Schaff[1], J. Dafoe[2], D. Boss[2], J.A. Boles[1]
[1]Montana State University, Bozeman, USA. [2]Montana State University, Havre, USA
(jennifer.thomson@montana.edu)

Fat deposition is crucial for both livestock producers and consumers. Producers determine harvest timing based on body composition or fatness, while consumers associate marbling with tenderness. USDA quality grades, determined by physiological age and marbling, provide insight into product palatability, though variation exists within the same grade. This study aimed to enhance the understanding of the regulation of fat deposition and muscle growth in relation to USDA quality grading. Fifteen commercial crossbred Angus Charolais steers were fed at the Northern Agricultural Research Station in Havre, MT, to endpoints representing USDA Standard, Select, or Choice quality grades (N=4 Choice, 6 Select, and 5 Standard). The study was conducted under the guidance of the Montana State University Agriculture Animal Care and Use Committee (Protocol #2020-A1231). Animals were harvested at a local state-inspected processing plant, where subcutaneous adipose tissue and Longissimus muscle and liver samples were collected at harvest and snap-frozen for RNA extraction. Carcass data were assessed by an experienced evaluator 24 hours postmortem. RNA was extracted from tissue samples and sequenced by a commercial sequencing lab using rRNA depletion methods, generating approximately 5 million mapped reads per sample. Differential gene expression analysis was conducted between quality grade classifications in all tissues, with gene enrichment analysis performed using the GO ontology and KEGG pathway databases. Statistical significance was determined at Bonferroni-adjusted $P<0.05$. A total of 2021 genes were differentially expressed in comparing Choice to Standard Adipose tissue. Upregulated pathways of note included the expected lipid transport and synthesis and activation of LXR/RXR, which suppresses lipid metabolism in hepatocytes and activates inflammatory mediators in macrophages. There were 1442 genes differentially expressed in the muscle tissue when comparing Choice to Standard tissues. The most enriched pathways in this gene set also included LXR/RXR activation, acute phase response signaling, and regulation of IGF, Insulin-like Growth Factor, transport and uptake. This indicates tissue inflammation and potential tissue injury. Further, there were 1113 differentially expressed genes in the liver tissue of Choice animals compared to Standard animals. Pathways of interest inhibit lipid metabolism in the liver and upregulate pathogen-induced cytokine storm signaling pathways. Taken together, these tissue indicators indicate systemic inflammation and insulin resistance similar to the chronic condition seen in metabolic syndrome in humans (Kawai, 2021). This study confirms previous work in our lab that has shown that adipose tissue and muscle tissue gene expression changes when fat is deposited, and that rapid fat deposition, as seen in animals with USDA Choice or higher grades, can trigger an acute inflammatory response (Thomson, 2022). This may provide insight into more precise management of fat deposition to increase productive efficiency.

References

Thomson, J.M., Schumacher, M.L., Boles, J.A., 2022. Animal - science proceedings 13, 296-297. https://doi.org/10.1016/j.anscip.2022.07.053
Kawai, T., Autieri, M.V., Scalia, R., 2021. American Journal of Physiology - Cell Physiology C, 375-391. https://doi.org/10.1152/ajpcell.00379.2020

Funding

Appreciation is expressed to USDA-NIFA Grant 2020-02663 and MONB00115 for funding this research.

Effect of walking on productive responses and metabolic profile of dairy cows

M. Carriquiry[1], D. Talmón[1], A. Jasinsky[2], P. Chilibroste[2], M.M. García-Roche[1]
[1]Facultad de Agronomía, UdelaR, Montevideo, Uruguay. [2]Estación Experimental Mario A. Cassinoni, Facultad de Agronomía, UdelaR, Paysandú, Uruguay (mcarriquiry@fagro.edu.uy)

Walking, particularly in grazing systems in which access to feed resources depends largely on animal locomotion, may represent a significant portion of total energy expenditure (Ribeiro et al., 1977) and thus, it could impact on nutrient partitioning of grazing cattle. Thus, the aim of this study was to evaluate the effect of walking on productive responses and metabolic profile of dairy cows. Twenty-four Holstein cows (110 ± 18 days in milk, 31.3 ± 4.1 kg/d of milk yield, 622 ± 58 kg of body weight (BW) and 2.9 ± 0.3 units of body condition score) were used in a complete randomized design with two treatments: cows that did not walk (NO_WALK; n=12) or that walked 8 km/day (WALK; n=12). Cows were housed in a compost barn (30 m from milking parlor) and were fed a total mixed ration ad libitum (48:52 forage to concentrate ratio; 41.5% DM, 15.5% CP and 34.1% NDF). During an adaptation period (days -7 to -1) all cows received the same diet but neither group walked (baseline). The experimental period lasted 12 days (days 1 to 12) during which WALK cows walked 4 km/d after morning milking and 4 km/d before afternoon milking (3.1 ± 0.2 km/h), while NO_WALK cows remained in the compost barn without access to feed or water. Heart rate (HR) was recorded continuously (day 1 to 12) and blood samples were collected at days -6, 6 and 12. Data were assessed as repeated measures with a model including treatment as fixed effect; means were considered to differ when $P<0.05$. Neither DM intake nor milk yield or milk components differed between treatments (25.7 ± 0.7 kg DM/d and 31.3 ± 2.3 kg/d of milk) but BW during the experimental period tended ($P=0.10$) to be affected by treatment as WALK cows maintained while NO_WALK cows gained BW (0.07 vs. 0.68 ± 0.3 kg of BW change, respectively). Daily HR average was similar between WALK and NO_WALK cows (91.8 ± 2.5 beats/min) but while walking was 1.3-fold greater for WALK cows. Plasma concentrations of glucose, non-esterified fatty acids, β-hydroxybutyrate, triglycerides and cholesterol did not differ between treatments. Although creatinine concentrations did not differ, plasma total protein decreased while urea concentrations increased for WALK than NO_WALK cows (73.3 vs. 82.6 ± 3.0 g/L and 5.9 vs. 5.2 ± 0.3 mmol/L). In agreement with our results, changes in plasma metabolites in dairy cows have been reported to quickly return to their baseline values after walking (Pearson and Archibald, 1989). This work would indicate that walking reduced partition nutrients towards BW gain and increased protein turnover probably in response to greater energy demands, leaving amino acids available to catabolism to be used for ATP production or as non-sugar gluconeogensis precursors.

References

Ribeiro, J.M., Brockway, J.M., Webster, A.J.F., 1977. Animal Production 25, 107-110.
https://doi.org/10.1017/S0003356100039118
Pearson, R.A., Archibald, R.F., 1989. Animal Science 48, 375-384.
https://doi.org/10.1017/S000335610004036

Funding

This research was partially founded by Comisión Académica de Posgrados (Universidad de la República, Uruguay) through a postgraduate scholarship awarded to D. Talmón and by the Comisión Sectorial de Investigación Científica (Universidad de la República, Uruguay; CSIC VUSP 2023 awarded to M. Carriquiry)

Required dietary digestible lysine : digestible crude protein ratio for optimal growth performance in weaned piglets fed diets restricted in crude protein content

E.J. Alderliesten[1], P. Bikker[2], A.J.M. Jansman[2]
[1]ForFarmers, Lochem, Netherlands. [2]Wageningen University & Research, Wageningen, Netherlands (evelien.alderliesten@forfarmers.eu)

Standardised ileal digestible (SID) lysine (LYS) requirements of weaned pigs may exceed 1.3 g SID LYS/MJ net energy (NE) according to a review of 41 dose-response studies by Goethals et al. (2025). Application of this high LYS level would require an equally as high level of dietary crude protein (CP). Since practical diets for weaned pigs are generally restricted in CP content to minimise digestive disorders, the authors suggest to apply the dietary SID LYS level in function of CP rather than NE. The present study was conducted to determine the optimal SID LYS/SID CP ratio for growth performance in weaned pigs from day 7 to 35 post weaning. In total 704 pigs (TN70 x Tempo; initial body weight 9.0 kg±1.35 kg) were allocated at day 7 post weaning to eight dietary treatments, with eight pens, each with a litter of 11 pigs, per treatment. The calculated dietary SID LYS/SID CP ratio increased from 4.8 to 10.3% in eight equidistant steps at constant dietary CP content of 180 g/kg, 9.5 MJ NE/kg and other essential amino acids at 105% of Dutch CVB-recommendations relative to the highest LYS level. Feed intake and average daily gain (ADG) were recorded in two-week periods. Ileal digestibility of CP and amino acids were determined in a parallel nitrogen balance study with ileal-cannulated pigs and used in the statistical analysis and estimate of SID LYS requirements. Data were analysed with ANOVA and broken line regression using Genstat with pen as experimental unit, dietary SID LYS/SID CP as fixed factor, and room as random factor. Feed intake and ADG increased ($P<0.001$) and FCR decreased ($P<0.001$) according to a linear-plateau relationship with increasing SID LYS/SID CP ratio in each two-week period and the total four-week period. In the total period, feed intake increased from 476 g/d at the lowest LYS level to a plateau of 688 g/d reached at 6.4% SID LYS/SID CP. Average daily gain increased from 274 g/d to a plateau of 536 g/d reached at 6.9% SID LYS/SID CP. Feed conversion ratio decreased from 1.74 to a plateau of 1.26 reached at 7.0% SID LYS/SID CP. Nitrogen retention in the digestibility study increased to a plateau of 22.0 g/d at 7.0% SID LYS/SID CP. These results indicate an optimum ratio of SID LYS/SID CP of 6.8-7.0% for maximum gain and feed efficiency in the diet of weaned pigs, with restricted CP content. This value is slightly lower than the theoretically derived ratio of 7.83% proposed by Goethals et al. (2025), and can be used as a preliminary recommendation when formulating low protein diets for weaned pigs.

Reference

Goethals, S., Bikker, P., Rijpert, J.H.M., Ampe, B., Spek, J.W., Millet, S., 2025. Animal 19, 101323. https://doi.org/10.1016/j.animal.2024.101323

Funding

This research was conducted within the public private partnership “Feed4Foodure-III; Sustainable animal nutrition in circular agri-food systems” (TKI-Agrifood LWV20203), partially funded by the Ministry of Agriculture, Fisheries, Food Security and Nature (project BO-55-001-015), and partly by “Vereniging Diervoederonderzoek Nederland”.

Optimizing early protein supply for robustness and health in fast- and slow-growing chicks

S. Métayer-Coustard[1], E. Cailleau-Audouin[1], T. Bordeau[1], B. Méda[1], F. Derouin-Tochon[1], E. Gambier[1], N. Même[2], M. Quentin[3], E. Pampouille[3], S. Tesseraud[1]
[1]INRAE, Université de Tours, BOA, 37380 Nouzilly, France. [2]INRAE, PEAT, 37380 Nouzilly, France. [3]Institut Technique de l'Aviculture, 37380 Nouzilly, France (sophie.tesseraud@inrae.fr)

The nutrition of young chicks is a delicate matter, as they switch from a maternal diet to an exogenous diet. However, the impact of protein supply in the first days of life is poorly known, even though early feeding is a key for chick robustness and health. This study aimed at investigating the effect of dietary crude protein (CP) content on different criteria linked to metabolism, robustness and health. Two experiments were performed in fast-growing (FG) and slow-growing (SG) chicks. Day-old male chicks received one of six diets differing by their CP content (FG: 17.5-22.5%; SG: 16.5-21.5%), formulated with the constraints currently used for amino acid (AA) balance and with similar energy and digestible lysine levels. Growth performance was regularly measured during the starter phase. Relative weights of *Pectoralis* muscles were determined on d5 and d10 (FG) and on d10 and d20 (SG), as were serum metabolites/biomarkers proposed by Travel *et al*. (2021). Chemical composition of the whole body was analyzed on d7 in FG and d15 in SG chicks. Reducing the dietary CP content impaired growth performance of both strains, particularly for the lowest CP diets. The relative weight of *Pectoralis* muscles was also significantly lower for the lowest CP diets at d10 in FG (17.5 and 18.5% CP) and d20 in SG (16.5% CP); these values did not differ between diets earlier (*i.e.*, d5 in FG and d10 in SG chicks). For both strains, blood glucose levels were unaffected by the reduction in CP content, whatever the age. Conversely, triglyceride levels increased linearly with CP reduction, with a diet × age interaction and an age effect (higher values for early measurements regardless of strain). Body fat content measured at d7 and d15 (FG and SG chicks, respectively) also increased linearly with CP reduction. This was not the case for body protein content, which remained unchanged whatever the strain. Reducing CP content strongly decreased uric acid levels, reflecting lower AA catabolism in low CP diets, particularly at early ages in both strains. These results are consistent with those for the biomarker linked to total antioxidant status (TAS). A positive correlation between TAS and uric acid was observed, partly explained by the antioxidant role played by uric acid. Haptoglobin, a marker of inflammation, was not modified by the diet, which may be linked to the good experimental conditions of our study, during which chicks were not challenged. In conclusion, our results showed an impact of reducing CP on performance, body composition, and some metabolites/biomarkers in both strains. While they provide a basis for optimizing early protein nutrition, further studies are needed by changing AA supply (AA balance, functional AA) and considering trade-off between productive and adaptive functions (challenged conditions).

Reference

Travel, A., Petit, A., Barat, P., Collin, A., Bourrier-Clairat, C., Pertusa, M., Skiba, F., Crochet, S., Cailleau-Audouin, E., Chartrin, P., Guillory, V., Bellenot, D., Guabiraba, R., Guilloteau, L.A., 2021. Frontiers in Veterinary Science 8, 759456. https://doi.org/10.3389/fvets.2021.759456

Funding

This research was supported by the French Ministry of Agriculture (CASDAR project PRESAJE).

Influence of genotype and parturition management on metabolic profiles in piglets differing in birth weight

H. Lickfett[1,2], M. Oster[1], A. Vernunft[1], H. Reyer[1], S. Görs[1], C.C. Metges[1], H. Bostedt[2], K. Wimmers[1,3]
[1]Research Institute for Farm Animal Biology (FBN), Dummerstorf, Germany. [2]Veterinary Clinic for Reproductive Medicine and Neonatology, Justus-Liebig-University, Gießen, Germany. [3]Chair of Animal Breeding and Genetics, Faculty of Agricultural and Environmental Sciences, University Rostock, Rostock, Germany (wimmers@fbn-dummerstorf.de)

The high pre-weaning mortality rate on commercial farms is a cause of concern and requires adaptations of breeding and management approaches for effective reduction. Piglets are highly vulnerable during the first few days *postnatum*, especially those with a low birth weight. In this study, neonatal adaptation was examined in relation to genotype, housing conditions, hormonally induced parturition, and birth weight category. In total, the neonates of 35 farrowing events of the breeds German Landrace (GL; parturition induction at day 115 of gestation or spontaneous farrowing) and German Saddleback sows (GS; parturition induction at day 112 of gestation) were examined, which were kept in crates (1.0 m^2) or pens (6.5 m^2) ($n = 144$). The piglets were categorised according to their birth weight as hypotrophic (800–1100 g), eutrophic (> 1100–1500 g), or hypertrophic (> 1500 g). Repeated blood samples were taken at 0.5-6.0 h, day 1, day 4, day 20 *postnatum* and one day after weaning. Analyses of plasma metabolites included albumin, ammonia, chloride, cortisol, creatinine, fructose, glucose, haptoglobin, inositol, insulin, lactate, non-esterified fatty acids (NEFA), sodium, total protein, triglycerides, triiodothyronine, urea and uric acid. All piglets showed an increase in total protein concentration ($P < 0.05$) during the first day as a result of colostrum intake. A Partial Least Squares Discriminant Analysis showed, that eutrophic and hypertrophic piglets had a similar metabolite profile ($P < 0.05$) and they were used as controls for downstream analyses. In contrast, hypotrophic piglets could be discriminated from their heavier littermates ($P < 0.05$). Hypotrophic neonates were more prone to exhibit a hypoglycaemia-hypothermia complex shortly after birth, which might be mitigated by the observed increased release of cortisol. Results further suggest a higher organ maturity of GS piglets compared to GL piglets due to improved ammonia and creatinine excretion. Also spontaneously born piglets might have a higher organ maturation and possibly more efficient protein utilisation compared to piglets from induced births as suggested by lower plasma glucose and lactate levels, but higher NEFA, total protein and urea levels ($P < 0.05$). Housing conditions during farrowing affected plasma glucose and inositol levels during the neonatal adaptation period, which could be due to differences in physical activity in farrowing pens compared to crates. Taken together, the piglet vitality and plasma metabolite dynamics during the suckling period are influenced by genetic background, parturition induction, housing conditions and birth weight class and reflect differences in tissue maturity and utilization of energy reserves.

Funding

This work was financially supported by the commercial Tönnies Forschung gGmbH.

Effects of maternal high- or low- protein intake on the sow reproductive performance, serum parameters, and offspring growth performance and protein metabolism

Y. Li[1,2], H.M. Hammon[3], C.C. Metges[3], N. Everaert[2]
[1]Shandong Agricultural University, Tai'an, China. [2]KU Leuven, Leuven, Belgium. [3]Research Institute for Farm Animal Biology, Dummerstorf, Germany (nadia.everaert@kuleuven.be)

Maternal nutrition during gestation can have lasting effects on offspring health. Our previous study found that piglets from sows fed a high crude protein (CP) diet during late gestation had lower serum urea levels at weaning compared to those from sows on a low CP diet. However, one week post-weaning, these piglets exhibited higher serum urea levels, suggesting maternal programming of nitrogen metabolism. In this study, we examined the impact of maternal CP intake on sow reproductive performance, serum biochemical indices, and piglet growth and protein metabolism. Twenty multiparous Landrace sows were assigned to two groups on day 84 of gestation, receiving diets with either 17% CP (high protein, HP; no added crystalline AA), or 12% CP (low protein, LP; crystalline AA added to meet AA requirements) for the last five weeks of gestation. Post-farrowing, all sows were fed the same lactation diet, and piglets were weaned at four weeks. Sow performance, CP digestibility and litter size were recorded. Blood and feces were collected one week before farrowing. After weaning, two male piglets per sow were selected and fed a standard diet until seven weeks. They were then housed individually for a five-day isotope tracer experiment, which included a single dose of [15N]-Gly to assess nitrogen metabolism. Blood samples were collected at weaning and at the end of the tracer experiment. Results showed no significant differences in sow body weight, backfat thickness, body condition score, reproductive performance, or CP digestibility between the two groups. However, HP sows had higher serum levels of albumin, alkaline phosphatase, glucose, and urea, and lower monocyte counts. Piglets from HP sows exhibited lower serum urea and glutamate dehydrogenase levels, higher red blood cell and hemoglobin counts at weaning, and increased aspartate aminotransferase, bilirubin, and non-esterified fatty acids by week seven. The rate of whole body protein synthesis (3.79 ± 0.33 *vs.* 3.88 ± 0.30 g N/(kg d)), rate of whole body protein breakdown (2.49 ± 0.32 *vs.* 2.55 ± 0.29 g N/(kg d)), fractional rate of protein synthesis (13.92 ± 1.23 *vs.* 14.25 ± 1.10 g N/d), fractional rate of protein breakdown (9.14 ± 1.17 *vs.* 9.12 ± 1.05 g N/d), and fractional rate of protein accretion (4.78 ± 0.19 *vs.* 4.88 ± 0.22 g N/d) did not differ significantly between the two groups (HP vs LP, mean ± SEM). The urea concentrations in the serum of sow and piglets were in line with our previous study. These findings suggest that a low-protein diet supplemented with crystalline amino acids during late gestation did not adversely affect sow reproductive performance or piglet growth, though it did alter certain blood parameters related to liver, muscle, and kidney functions, while the tracer experiment did not confirm an altered protein metabolism of piglets.

Funding

This work was supported by the PIGWEB project which has received funding from European Union's Horizon 2020 research and innovation program under Grant Agreement No 101004770.

Dietary supplementation with functional amino acids improves energy efficiency of salmonella-challenged growing pigs under poor housing conditions

D.A. Marçal[1], G.A.C. Valini[1], P.R. Arnaut[1], I. França[1], M.T. Ortiz[1], M.J.K. Oliveira[1], A.D.B. Melo[1], P.H.R.F. Campos[2], A.M. Veira[1], A.Z. Fraga[1], J.K.K. Htoo[3], H.G. Brand[4], L. Hauschild[1]
[1]Department of Animal Science, School of Agricultural and Veterinarian Sciences, São Paulo State University, Jaboticabal, Brazil. [2]Department of Animal Science, Universidade Federal de Viçosa, Viçosa, Brazil. [3]Evonik Operations GmbH, Hanau, Germany. [4]Evonik Brasil Ltda., Sao Paulo, Brazil (luciano.hauschild@unesp.br)

Pigs under sanitary challenge (SC) shifts nutrients and energy from tissue deposition to immune responses (Pastorelli et al. 2012). Functional amino acids (FAA) play key roles in defense mechanisms such as gut barrier integrity, immune protein synthesis, and antioxidative status. This study aimed to evaluate the energy balance of growing pigs fed FAA-supplemented diets under SC. Eighty female pigs (25.5 ± 2.38 kg) were blocked by BW and randomly assigned to one of four treatments in a 2×2 factorial with two housing conditions [(GOOD) or challenged with *Salmonella* Typhimurium (ST) under poor housing condition (STPOOR)] and two diets [control (CN), with AA profile according to NRC (2012) or supplemented (AA+), with 20% higher Thr, Met+Cys, Trp]. At d0, pigs in STPOOR were inoculated with ST (2×10^9 CFU/mL) and manure from a commercial pig farm was spread on the floor. During the study, STPOOR was not cleaned while GOOD was cleaned twice a day. Diets were provided through automatic and intelligent precision feeders. Individual BW and body composition were measured on d0 and 28. Energy retained as protein and lipid were estimated considering body protein and lipid contents of 23.8 and 39.6 MJ/kg, respectively. Heat production (HP) was calculated subtracting total energy retained from metabolizable energy (ME) intake. Metabolic HP was calculated as the ratio between HP and $BW^{0.60}$, and energy efficiency as the ratio between energy retained and ME intake. Data were subjected to variance analysis including housing condition, diet and their interaction. Initial BW was used as covariate. P-values ≤ 0.05 were significant and <0.10, tendencies. Pigs under STPOOR showed a lower ($P \leq 0.05$) ME intake, energy retained as protein and lipid and energy efficiency than GOOD group. Interaction between housing condition and diet was observed for energy efficiency ($P \leq 0.05$) in which in GOOD there was no diet effect, while in STPOOR pigs fed AA+ were more efficient than CN group, and furthermore were as efficient as pigs in GOOD. Trends for interaction were observed for energy retained as protein ($P=0.07$) and energy retained ($P=0.07$). Pigs fed CN in STPOOR retained less energy as protein than pigs fed AA+, which had similar values as in GOOD. For energy retention, pigs in GOOD retained more than in STPOOR with no diet effect, while in STPOOR, pigs fed AA+ retained more energy than CN group. Pigs under STPOOR had lower ME intake and energy conversion to lipid. Finaly, isolated effects of diet ($P \leq 0.05$) were observed for HP and metabolic HP. Pigs fed AA+ lost less energy as heat than CN. Our results show that the SC affected the energy efficiency, but the dietary FAA supplementation was able to decrease energy dissipation and maintain energy efficiency.

Reference

Pastorelli, H., van Milgen, J., Lovatto, P., Montagne, L., 2012. Animal 6, 952-961. https://doi.org/10.1017/S175173111100228X

Funding

This work was supported by the São Paulo Research Foundation (2018/15559-7, 2019/10843-1, 2020/15797-5 and 2023/08305-7), Brazilian National Council for Scientific and Technological Development (CNPq, grants number 308779/2023-1, 408287/2021-7 and 442675/2023-2), Evonik Operations GmbH, and Seara Alimentos - JBS Foods Brazil.

Net energy use efficiency of weanling pigs is enhanced by diluting diets with dietary fiber

T.G. Hulshof[1], R. Yao[2,3], G.P.J. Janssens[2], A. Cools[2], M. Merckx[2], D. Maes[3], H.M.J Van Hees[1,2]
[1]Trouw Nutrition R&D, Boxmeer, Netherlands. [2]Department of Veterinary and Biosciences Ghent University, Merelbeke, Belgium. [3]Department of Internal Medicine, Reproduction and Population Medicine Ghent University, Merelbeke, Belgium (tetske.hulshof@trouwnutrition.com)

Dietary fiber (DF), being almost completely indigestible, can help gastrointestinal tract maturation, thereby, reducing post-weaning diarrhea which is important for weanling pigs. The study aimed to determine the effect of DF inclusion in weanling pigs’ diets on growth performance and energy use efficiency during 39-days post-weaning. The experiment was designed as split-plot with 3 treatments pre-weaning (N=36 litters) followed by 2 treatments post-weaning (N=72 pens). A pre-wean effect was absent and, therefore, only the post-weaning phase is described. Post-weaning, 6 pigs per litter were assigned to the CONTROL diet consisting of a basal weaner feed + 28% wheat+barley (50:50 ratio; NE 2610 kCal/kg, DF 15.0% (AOAC method 991.43); CONTROL_W). Six other pigs were assigned to the GRASS diet consisting of a basal weaner feed + 28% grass pellets (NE 2235 kCal/kg, DF 28.7%; GRASS_W). On d14 post-weaning, pigs transitioned onto a link diet consisting of a basal link feed added with 13% wheat+barley (50:50 ratio; NE 2465 kCal/kg, DF 19.3%) or grass pellets (NE 2290 kCal/kg, DF 25.6%) for CONTROL_W and GRASS_W, respectively. Diets were isonitrogenous (CP 17%; SID Lys 1.12%). Growth performance, NE intake and NE use efficiency (gain/NE intake) were assessed on d14 and 39. Data were analyzed (PROC MIXED) as a split-plot design, with pre-wean litter as the main plot and post-wean pen as a split plot. During d0-14, GRASS_W had a lower growth performance compared to CONTROL_W, that is average daily gain (182 vs 227 g/pig/d; $P<0.001$), average daily feed intake (221 vs 262; $P<0.001$), gain:feed ratio (0.83 vs 0.87; $P<0.001$), NE intake (494 vs 685 kCal/d; $P<0.001$), and bodyweight at d14 (9.4 vs 10.1 kg; $P<0.001$). During the second feed phase, where DF inclusion was reduced, average daily gain (511 vs 513 g/pig/d) and NE intake (1735 vs 1778 kCal/d; $P=0.136$) were similar but average daily feed intake was higher for GRASS_W than for CONTROL_W (758 vs 722 g/pig/d; $P=0.006$) resulting in a lower gain:feed (0.68 vs 0.71; $P<0.001$). The difference in bodyweight seen at d14 (0.7 kg) was sustained until the end of the trial (22.2 vs 22.9 kg for GRASS_W vs CONTROL_W, respectively; $P=0.009$). In both feed phases, the NE use efficiency was higher for GRASS_W than for CONTROL_W: 0.37 vs 0.33 g/kCal for phase 1 ($P<0.001$) and 0.30 vs 0.29 g/kCal for phase 2 ($P<0.001$). In conclusion, diluting weanling pigs' diets with DF increases energy use efficiency during the complete post-weaning phase. However, the DF level of the diet fed during the first 2-weeks post-weaning should be reconsidered since a DF of 25.6% hampered feed intake and, therefore, growth performance during the complete nursery phase.

Funding
This research was funded by Trouw Nutrition.

Comparison of fat and fiber driven reductions in dietary energy density on voluntary energy intake and energy partitioning in growing pigs

P. Aymerich[1,2], T. Lenke[3], J. Coma[1], H.M. Hammon[3]
[1]Vall Companys Group, Lleida, Spain. [2]Danisco Animal Nutrition & Health (IFF), Oegstgeest, Netherlands.
[3]Research Institute for Farm Animal Biology (FBN), Dummerstorf, Germany (pau.aymerich@iff.com)

Growing pigs increase feed intake when dietary net energy (NE) is reduced to reach similar intake (Quiniou and Noblet, 2012) but there might be limitations in this compensation (Aymerich et al., 2020). The present experiment aimed to compare the effect of fat and fiber driven energy reductions on energy intake and partitioning in growing pigs. A total of 64 growing gilts [German Landrace] with initial BW of 44.7±8.83 kg were used in a 4 treatment factorial design. Each week, 4 gilts were randomly allocated to one of the 4 treatments. Pellet diets were based on corn, wheat, barley, soybean meal. Rapeseed oil (RSO) and wheat middlings (WM) were used to change dietary NE. In all treatments the standardized ileal digestible lysine (SID Lys) to NE ratio was kept constant. Treatments were: T1) high energy, 4.4% RSO and no WM; T2) medium energy without RSO and WM; T3) with 4.4% RSO and 24.5% WM; T4) low energy without RSO and with 24.5% WM. From day 1-15 gilts were fed *ad libitum* in individual pens. At day 15 each pig was moved to the calorimetry chambers. There pigs were fed *ad libitum* from day 16 to 17 (AL) and restricted at 85% of the minimum *ad libitum* NE intake in that period between day 17 and 18 (RE) to have equal NE intake between treatments. Feed intake was daily measured and pigs were weighed at days 1, 7, 15, 18 and 21. In the chambers, total O_2 consumption and CO_2 production were measured every 6 minutes and used to calculate net disappearance rates of carbohydrates and lipids. Respiratory quotient (RQ) was calculated dividing the production of CO_2 by the consumption of O_2. All data was analyzed in mix model including period as random effect and fat, fiber and their interaction as fixed effects. From day 1 to 15, reducing RSO level increased ADFI (+0.15 kg; $P<0.010$) but did not significantly modify NE intake (-0.16 MCal/d; $P>0.100$). Contrarily, increasing WM inclusion did not significantly increase ADFI (+0.05 kg; $P>0.100$) but significantly reduced NE intake (-0.38 MCal/d; $P<0.01$). No significant interaction was reported between the two factors ($P>0.1$). In both the AL and RE, reducing RSO increased RQ ($P<0.01$) as a result of increased carbohydrate disappearance ($P<0.01$) and reduced lipid disappearance. Contrarily, increasing WM reduced RQ ($P<0.05$) only in the RE as a result of a tendency for lower lipid disappearance ($P<0.1$). In conclusion, this study confirmed that there are relevant differences when dietary energy density is reduced by reducing fat inclusion or increasing high fiber ingredients, the first one will greatly modify source of energy used whereas the use of high fiber could limit energy intake.

References

Aymerich, P., Soldevila, C., Bonet, J., Gasa, J., Coma, J., Solà-Oriol, D., 2020. Animals 10(9), 1638.
https://doi.org/10.3390/ani10091638
Quiniou, N., Noblet, J., 2012. Journal of Animal Science 90(12), 4362-4372.
https://doi.org/10.2527/jas.2011-4004

Timothy hay inclusion into growing finishing pigs diets alters the energy metabolism and growth rate resulting in leaner pigs

B. Llorens[1,2], C. Pomar[1], M.P Létourneau-Montminy[2], R.M Petri[1], N. Diether[3], A. Remus[1]
[1]Agriculture and Agri-Food Canada, Sherbrooke, Canada. [2]University Laval, Quebec, Canada. [3]Dalhousie University, Halifax, Canada (aline.remus@agr.gc.ca)

Identifying alternative feed ingredients that reduce feed-to-food competition will enhance pork sustainability. Timothy grass shows promise as a nutritious ingredient for growing pigs, but further knowledge is required. This study evaluated the effects of timothy meal inclusion (TI) on growth, protein and lipid deposition (PD and LD, respectively) in two feeding systems (FS): conventional group 3-phase feeding (GPF) and individual precision feeding (IPF). Fifty-six pigs [37.4 ± 0.7 kg of body weight (BW)] were randomly assigned to dietary treatments based on FS and TI (with=1, without=0): GPF0, GPF1, IPF0, and IPF1. In GPF1, TI levels were 8%, 16%, and 22% across three growing phases. In IPF1, TI was gradually increased based on BW to match GPF1's overall inclusion rate. Average daily feed intake (ADFI) was recorded daily, BW weekly, with body protein and lipid masses estimated via dual X-ray absorptiometry at the beginning and end of each growing phase (i.e., on days 1, 21, 42, and 63). On the same days, fecal short-chain fatty acids (SCFA), non-esterified fatty acids (NEFA), and basal plasma amino acids samples were collected after 10h fasting; only the relevant amino acid changes will be presented. Data was analyzed as repeated measures with FS, TI, sex, time, and their interactions as fixed effects and the individual as a random effect. No differences in ADFI were found among treatments, but digestible energy intake decreased 9% ($P < 0.01$) with TI and 8% ($P= 0.06$) with IPF. Overall, TI reduced average daily gain (ADG) by 9% ($P < 0.01$) independent of FS. TI decreased PD by 4% ($P < 0.01$) without affecting final body protein mass, whereas LD decreased by 22% ($P < 0.01$). Fecal acetate and propionate concentrations increased by 4% ($P < 0.01$) with TI, independent of FS. NEFA concentrations in plasma tended ($P = 0.07$) to increase by 35% with TI, but decreased ($P < 0.01$) over time. Plasma threonine and serine decreased by 7% ($P < 0.01$) with TI. This may indicate increased utilisation of Thr and Ser by intestinal tissues due to greater endogenous losses for desquamation with higher fiber intake (Lien et al., 2001). Altogether, TI increased lipid mobilization as shown by the NEFA values throughout growth. However, a decrease in plasma NEFA over time suggests a partial change in energy metabolism from glucose to SCFA as an energy source. Although TI slowed growth and delayed market weight, it led to more consistent PD, especially influencing ADG composition in phase 3 for pigs fed timothy. The 7% lower final BW was mainly due to reduced LD, with no change in final body protein mass regardless of FS, resulting in a higher lean percentage. Likely, a longer study would allow to better show evidences of TI energy use over time.

Reference

Blank, B., Eva, S., Susenbeth, A., 2012. Archives of Animal Nutrition 66, 86-101. https://doi.org/10.1080/1745039X.2012.663669

Funding

This work was funded by the Canadian Swine Cluster 4 and AAFC.

Adaptation period length influences protein and amino acid requirement estimates: a reanalysis of human and swine data

C.D. Ramirez-Camba[1], C.L. Levesque[2], P.E. Urriola[1]
[1]University of Minnesota, St. Paul, Minnesota, USA. [2]South Dakota State University, Brookings, South Dakota, USA (ramir643@umn.edu)

Establishing an appropriate adaptation period in studies of protein and amino acid (AA) requirements is critical to ensuring that estimates reflect true physiological needs rather than transient responses. In nitrogen balance (NB) studies, a 7-day adaptation period is common, whereas Indicator Amino Acid Oxidation (IAAO) studies often use only 2 days. These differing protocols raise the question of when transient responses have dissipated, and a new steady state is reached. In our study, we reanalyzed data from studies on human protein requirements and contrasted these findings with available swine data to identify parallels and gaps. We employed the linear-logistic model (Ramirez-Camba & Levesque, 2023), which identifies two inflection points: PDmax, representing the intake that maximizes protein deposition, and MSmax, indicating the intake associated with improved metabolic status and potential health benefits. Data for this analysis were drawn from four systematic reviews identified in February 2024 across PubMed, Scopus, and Web of Science. These reviews encompassed 83 studies conducted between 1951 and 2020 with experimental periods ranging from 3 to 21 days. Our model allowed us to calculate protein levels corresponding to PDmax and MSmax in 13 IAAO datasets and 24 NB datasets; the remaining datasets did not span a sufficient range of dietary protein to capture both responses. Our results show that protein retention levels corresponding to both PDmax and MSmax decrease quadratically as the adaptation period increases ($P < 0.001$), implying that protein requirement estimates decline with extended adaptation. Experiments using the IAAO method provided subjects with higher dietary protein—ranging from 0.10 to 3.51 g/(kg body weight·day)—whereas NB experiments used lower levels, ranging from 0.04 to 1.33 g/(kg·day). When protein intake is reduced, the body progressively downregulates certain metabolic processes to conserve nitrogen (Young & Marchini, 1990). Our results suggest that metabolic equilibrium requires approximately 3 days at 1.30 g/(kg·day) and 10 days at 0.54 g/(kg·day), regardless of the measurement method. The reanalysis of swine data shows that protein and AA intake levels corresponding to PDmax and MSmax vary as the experimental period progresses, suggesting shifts in protein and AA utilization that may reflect metabolic adaptations with as-yet-unknown consequences. Our findings suggest that protein and AA requirements are "moving targets" influenced by experimental design. If we consider that the standard adaptation period for AA and protein requirement studies in animal species such as swine is 7 days, our estimates may reflect protein demands of animals that have already downregulated important metabolic functions. Our work emphasizes revisiting adaptation durations to develop nutritional recommendations that optimize health and performance. Furthermore, our analysis underscores the limitations of the conventional linear-plateau model in requirement research, demonstrating that the linear-logistic model more effectively captures dynamic metabolic changes.

Reference

Ramirez-Camba, C.D., Levesque, C.L., 2023. Animals 13, 1708. https://doi.org/10.3390/ani13101708
Young, V.R., Marchini, J.S., 1990. The American Journal of Clinical Nutrition 51, 270–289. https://doi.org/10.1093/ajcn/51.2.270

Funding

The National Pork Board through the Real Pork Trust Consortium

Effects of time-restricted feeding on circadian rhythms of activities of membrane digestive enzymes of intestine and concentrations of serum essential amino acids and glucose in weaned piglets

T. Haruguchi, H. Kamoshima, H. Matsui, M. Katsumata
Azabu University, Sagamihara, Japan (m-katsumata@azabu-u.ac.jp)

We've been conducting research on chrono-nutrition to improve pig performance. Currently we are evaluating the effects of time-restricted feeding (TRF). Because we predicted that TRF would affect circadian rhythms of the absorption of essential amino acids and glucose, we aimed to clarify how TRF affects the circadian rhythms of membrane digestive enzymes in the small intestine, as well as the serum concentrations of essential amino acids and glucose in weaned pigs. Four-week-old barrows were used in this study. The feeding trial included eight replications of four light-restricted and four dark-restricted feedings. The feeding trials were conducted for three weeks. The pens were bright from 7:00 to 19:00 (12 h bright: 12 h dark). From 7:00 to 19:00, pigs in the light-restricted group had free access to their diet, while the pigs that were given dark-restricted feeding had free access to their diet from 19:00 to 7:00. After three weeks of restricted feedings, samples of jejunum, ileum and serum were taken at 3:00, 9:00, 15:00, and 21:00 to measure the activities of maltase and aminopeptidase N, as well as the concentrations of essential amino acids and glucose. TRFs did not affect the activities of maltase or aminopeptidase N in the jejunum or ileum. Concentrations of essential amino acids in the serum were higher at 09:00 than at 3:00 for all amino acids in the light-restricted feeding group, and higher at 21:00 than at 15:00 for all amino acids in the dark-restricted feeding group. Effects were detected for five essential amino acids including lysine and threonine ($P<0.05$)—start of eating triggered the increases in concentrations of amino acids regardless of feeding restriction method. In contrast, serum glucose concentrations were higher at 15:00 and lower at 21:00 ($P<0.05$) in the dark-restricted feeding group. There were no significant effects of the time of sampling in the light-restricted feeding group, but mean values were higher at 3:00 and lower at 9:00. In other words, as in the dark-restricted feeding group, a higher peak occurred four hours before feeding began. This presentation will cover the effects of TRF on the growth performance and circadian rhythms of mRNA expression of SGLT1 and GLUT2 in the ileum and jejunum. Mechanism that affecting circadian rhythms in serum glucose concentrations remains unknown. It is possible that the pigs' anticipation of feeding time impacted the regulatory mechanisms of blood glucose concentrations. We are planning to conduct experiments to clarify these regulatory mechanisms.

Funding
This study was supported by JSPS KAKENHI, Grant/Award Number: 21K05910.

Dietary threonine requirement for juvenile Nile tilapia

J.B.K. Fernandes[1], R.S.B. Sayao[1], T.S. Oliveira[1], R.S. Romaneli[1], H.J. Silva[1], N.K. Sakomura[2]
[1]Aquaculture Center of the São Paulo State University (CAUNESP), Jaboticabal, Brazil. [2]São Paulo State University (UNESP), School of Agricultural and Veterinary Sciences, Jaboticabal, Brazil (jb.fernandes@unesp.br)

Threonine, an essential amino acid for fish, plays an important role in growth, including body protein deposition and intestinal mucosa maintenance. Among traditional ingredients in commercial diets for Nile tilapia, threonine is the third limiting amino acid, highlighting the need to evaluate its dietary requirement to optimize feeding strategies. This study aimed to determine the dietary threonine requirement for juvenile Nile tilapia. Seven dietary treatments with increasing levels of total threonine (3.1, 6.3, 9.4, 12.5, 15.6, 18.8, and 21.9 g/kg) were evaluated, using four replicates per treatment. Diets were formulated based on the dilution method. A total of 560 tilapias (initial weight: 7.47 ± 0.03 g) were randomly distributed into 180-liter fiberglass tanks (20 fish per tank) in a recirculating water system equipped with a heat exchanger (28.37 ± 0.83 ºC) and constant aeration via radial blower (5.5 ± 1.0 mg/L). Body weight gain and body protein deposition were analyzed using polynomial regression. A quadratic effect was observed for both parameters, with optimal dietary threonine levels of 15.3 and 15.7 g/kg, respectively. Feed intake also exhibited a quadratic response. Body fat content, expressed as a percentage, remained stable up to the 12.5 g/kg threonine treatment but showed a linear reduction from 15.6 g/kg onward. Therefore, the dietary threonine requirement for maximum weight gain was 15.3 g/kg (6.03% of dietary protein), while the requirement for maximum protein deposition was 15.7 g/kg (6.19% of dietary protein).

Funding

The research was funded by the São Paulo State Research Foundation (FAPESP, Grant No. 2024/12857-8)-Brazil and Coordination for the Improvement of Higher Education Personnel – Brazil (CAPES) – Financial Code 001.

Feeding adult, male cats titrating protein diets 110-205% of National Research Council recommended allowance altered respiratory quotient but not energy expenditure and heat increment of feeding

S.F. Anan[1], D.J. Seymour[1,2], E. Labussière[3], A.K. Shoveller[1]
[1]Department of Animal Biosciences, University of Guelph, Guelph, Canada. [2]Ruminant Research Centre, Trouw Nutrition R&D, Boxmeer, Netherlands. [3]Pegase, INRAE, Saint-Gilles, France (sanan@uoguelph.ca)

Evaluation of dietary energy content within the pet food industry is based on digestible (DE) and metabolizable energy (ME) which accounts for fecal and urinary losses (de Oca et al., 2017). Quantifying net energy (NE) content of diets for domestic cats would help to better understand how macronutrient composition affects available energy after accounting for all energetic losses in obligate carnivores (Asaro et al., 2019). This study was designed to assess the NE content of feline diets increasing in crude protein (CP) content in exchange for nitrogen free extract (NFE) by calculating DE, ME and heat increment of feeding (HIF) after fecal, urine, and respiratory gas collections and measures, respectively. We hypothesized that increasing CP would increase energy expenditure (EE), HIF and decrease respiratory quotient (RQ). Two diets were formulated that provided CP at 110% (22% CP DM) and 205% (41% CP DM) of National Research Council (NRC) recommended allowance. These diets were combined to achieve four additional intermediate treatments (CP = 25%; 26%; 29%; 36% DM). Twelve cats were assigned per treatments in a 6×6 Latin square design and fed for a 14-day period. Actigraphs© were placed on d11 to track physical activity and on d13 cats were placed in calorimetry chambers specifically designed for domestic cats for total collection of feces, urine and oxygen (VO_2) and carbon dioxide (CO_2) exchange measurements over a 24-h period. EE, total energy expenditure (TEE), RQ and HIF were statistically analysed using SAS and the GLIMMIX procedure and repeated measures analysis were conducted for EE, TEE and RQ. HIF was estimated from area under the curve for TEE using linear trapezoidal rule. An effect of diet was observed for EE ($P = 0.05$), TEE ($P = 0.05$) and RQ ($P < 0.05$). Cats consuming the highest protein diet (41% CP) had the greatest EE (36.9kcal/kg BW) and TEE (180.4kcal/day). Nevertheless, the lowest EE (34.2kcal/kg BW) and TEE (168.0kcal/day) was observed within intermediate diet (25% CP) rather than the lowest protein diet (22% CP = 34.8kcal/kg BW; TEE = 168.9kcal/day). As expected, RQ was greatest in cats fed the lowest protein diet (22% CP EE = 0.83), while the highest protein diet resulted in the lowest RQ observed (41% CP = 0.80). There was an effect of time for all calorimetry outcomes ($P < 0.05$), but diet×time interactions were non-significant ($P > 0.05$). There was no effect ($P > 0.05$) of diet observed for immediate postprandial (4-h) HIF (10.7-13.2kcal/100g DM) or total postprandial (22-h) HIF (49.1-62.3kcal/100g DM). The RQ response demonstrates metabolic flexibility to shifting macronutrient utilization with increasing CP content in diets for cats. However, EE remained similar across diet levels due to no changes being observed in diet-induced thermogenesis which did not affect TEE overall in cats fed to maintenance.

References

de Oca, R.R., Valdiviezo, S.G., Jones, K.B., Dierenfeld, E.S., Simpson, C.F., 2017. Animal Feed Science and Technology 228, 91–101. https://doi.org/10.1016/j.anifeedsci.2017.04.004
Asaro, N.J., Seymour, D.J., Mansilla, W.D., Cant, J.P., Zijlstra, R.T., Berchtold, K.D., 2019. PLOS ONE 14, e0218173. https://doi.org/10.1371/journal.pone.0218173

Funding

The project was funded by Natural Sciences and Engineering Research Council of Canada (NSERC) Alliance grant and Petcurean Pet Nutrition. Additionally, experimental diets used in this research were formulated and manufactured by Petcurean.

Valine requirements of young adult and senior Labrador retrievers

S.M. Dickerson, C.L. Timlin, P. Skaggs, F.B. Mccracken, C.N. Coon
Four Rivers Kennel, Walker, USA (ccoon@uark.edu)

As the population of senior dogs continues to grow, there is a rising demand for age-specific diets that cater to their unique nutritional needs. However, research on the precise amino acid requirements of aging canines remains limited. Investigating these requirements is crucial, as it can guide the development of tailored dietary formulations for this life stage. Valine is an essential branched-chain amino acid (BCAA) that plays several crucial physiological roles, including synthesis of proteins essential for muscle growth, repair and maintenance, energy production, muscle metabolism and recovery, CNS function and neurotransmitter regulation, immune system support and glucose regulation. In this study, the indicator amino acid oxidation (IAAO) technique (Elango et al., 2008) was used to determine the minimum requirements for valine (Val) in young adult and senior Labrador retrievers. The indicator amino acid oxidation (IAAO) method is based on the concept that when 1 indispensable amino acid (IDAA) is deficient for protein synthesis, then all other IDAA, including the indicator amino acid, will be oxidized. After 2-day adaptation to Valine-adequate basal diet (Valine= 0.80% dry matter), dogs underwent individual IAAO studies. In brief, all dogs were randomly fed one of six test diets with varying levels of valine ranging from deficient to sufficient (final Val content in experimental diets was 0.349, 0.389, 0.429, 0.703, 0.753 and 0.803 % dry matter). Test diet was divided into 13 equal meals; at the 5th meal, a tracer amino acid was supplied (a bolus L-[1-^{13}C]-phenylalanine based on body weight was first given, followed by [1-^{13}C]-Phe doses every thirty minutes spanning a four-hour period), and breath samples were collected via respiration mask every thirty minutes. CO_2 production was determined via indirect calorimetry and production of $^{13}CO_2$ at each dose was measured by enrichment of ^{13}C in breath samples via isotope ratio mass spectrometry (IRMS). Results for IRMS data were converted to atom percent excess (APE) and analyzed using segmented linear regression. The Four Rivers mean and population requirements for valine were 287.5 ± 7.2 mg/MJ ME (mean ± 2SD) for young adults and 263.4 ± 12.2 mg/MJ ME (mean ± 2SD) in senior dogs, which is slightly higher than the National Research Council's minimum requirement and in line with their recommended allowance. Expressed in terms of requirements per lean body mass, we find a requirement of 11.7 mg/MJ ME/kg lean body mass for young adults, and 14.7 mg/MJ ME/kg lean body mass for senior dogs. As the pet food industry offers more specialized diets for aging canines, updating the amino acid requirements for such animals is increasingly important.

Reference

Elango, R., Ball, R.O., Pencharz, P.B., 2008. The Journal of Nutrition 138, 243–246. https://doi.org/10.1093/jn/138.2.243

Funding

The valine requirement for Labrador retrievers was funded by Four Rivers Kennel & Canine Senior Center, Walker, MO, USA

No different amino acid utilisation efficiency of fast- and slow-growing turkeys when fed organic diets reduced in crude protein up to day 28

W. Siegert[1], A. Sünder[1], S. Göppel[2], C. Lambertz[3], G. Bellof[2]
[1]University of Göttingen, Göttingen, Germany. [2]Weihenstephan-Triesdorf University of Applied Sciences, Freising, Germany. [3]Research Institute for Organic Agriculture (FiBL), Witzenhausen, Germany (wolfgang.siegert@uni-goettingen.de)

Meeting the recommendations of first-limiting amino acid concentrations in diets for organically farmed turkeys requires high crude protein levels in the feed, which also means excessive supply of other amino acids. Amino acid supply allowing for submaximal growth increased the utilisation efficiency (g accretion/g intake) of amino acids to a different extent between two genotypes of growing poultry (Fatufe *et al.* 2004). Therefore, this study investigated the trade-off between submaximal growth due to marginal amino acid supply on the amino acid utilisation efficiency in turkeys and whether this differs between fast- and slow-growing strains. Male hatchlings of the fast- and slow-growing turkey strains B.U.T.6 and Auburn, respectively, were fed according to the EU organic farming regulations (EU Regulation 2018/848) in a total of 18 floor pens on litter up to day 28 post-hatch (Göppel *et al.* 2022). Each pen comprised 20 animals at the begin of the experiment. Three pens per strain were assigned to one of three diets. Diets were formulated at 100%, 90%, and 80% of the Aviagen recommendations for lysine and methionine concentrations. Concentrations of all amino acids decreased when dietary lysine and methionine was reduced. Dietary crude protein thereby decreased from 326 to 264 g/kg. Growth and feed intake were determined. Hatchlings and two birds closest to the respective group weight on day 28 were euthanised and contents of the digestive tract were removed. Birds were homogenised, freeze-dried, and analysed for amino acids. Results were statistically analysed by two-way analysis-of-variance. No interaction between strain and diet was significant. Empty body weight gain was lower in Auburn compared to B.U.T.6 birds (0.91 vs. 1.11 kg; $P<0.001$) and decreased with dietary amino acid reduction (1.09 to 0.91 kg; $P<0.001$). Treatment effects on the accretion of crude protein and all amino acids were similar to empty body weight gain responses. The efficiency of crude protein and essential amino acid utilisation was not different between strains ($P\geq0.09$). The utilisation efficiency of crude protein and all amino acids increased with dietary amino acid reduction ($P<0.05$) with values of 45% to 52% for crude protein, 65% to 75% for lysine, 51% to 60% for methionine, and 50% to 60% for threonine ($P<0.001$). Among all amino acids, the utilisation efficiency was highest for glycine (79% to 92%; $P<0.001$). The results did not suggest a different utilisation of ingested amino acids between the fast- and slow-growing turkey strains under study. This indicated that the trade-off between growth and protein utilisation was similar despite different amino acid requirements for maintenance. Hence, nitrogenous emissions and resource input may be reduced if longer growth periods are accepted. An endogenous glycine production from precursors may have contributed to the very high utilisation efficiency.

References

Fatufe, A.A., Timmler, R., Rodehutscord, M., 2004. Poultry Science 83, 1314–1324. https://doi.org/10.1093/ps/83.8.1314
Göppel, K., Gude, H., Niemeyer, J., Pleickhardt, K., Winkelmann, M., Zebühr, Y., 2022. European Poultry Science 86, 10. https://doi.org/1399/eps.2022.362

Funding

The Federal Ministry of Food and Agriculture (BMEL) under the grant number 2819OE057.

Evaluation of the inclusion of ultra-processed foods in piglet and pig diets on liver and plasma profiles through omics-based approaches

M. Manoni[1], A. Altomare[2], S. Nonnis[1,3], M. Tretola[4], S. Mazzoleni[1], A. Luciano[1], E. Pacifico[1], G. Bee[4], G. Tedeschi[1,3], G. Aldini[2], L. Pinotti[1,3]
[1]DIVAS Department, University of Milan, Lodi, Italy. [2]DISFARM Department, University of Milan, Milan, Italy. [3]CRC I-WE, University of Milan, Milan, Italy. [4]Agroscope, Posieux, Switzerland (michele.manoni@unimi.it)

Ultra-processed foods (UPF) are known for their unbalanced nutrient profile and for being associated with unfavorable health outcomes in human. However, UPF are currently dominating global food systems, generating high amounts of waste along the food chain. Recycling UPF as feedstuff represents a way for reducing UPF waste and for replacing human-edible grains to reduce feed-food competition, making livestock production more sustainable. Monogastric such as pigs are the best candidates for consuming UPF waste given their human-like digestive tract. In a previous trial, 36 post-weaning piglets (28 day old, 6.5 ± 1.1kg) were assigned to three isoenergetic and isonitrogenous diets for 42 days: i) CTR, control (common grains), ii) salty (SA), iii) sugary (SU). For SA and SU diets, the 30% of grains was replaced with former food products (FFP), which are food leftovers falling in the UPF category. The same experiment was conducted on 36 growing pigs (22.4 ± 1.7 kg) for 110 days until slaughter (110 ± 3 kg). After observing that FFP inclusion did not cause any detrimental effect in both cases, we conducted an in-depth evaluation of the metabolic status of FFP-fed animals using omics-based approaches, focusing on plasma metabolome in piglets, and liver proteome and plasma peptidome in pigs. Mass spectrometry-based untargeted approaches were performed on plasma samples collected from piglets at trial onset and end and on liver tissue samples collected from pigs after slaughter. The piglets' plasma metabolome was more affected by time than SA and SU diets (36 vs 2 metabolites modulated, respectively), with only caffeine and theobromine significantly increased in SA and SU groups compared to CTR. Caffeine and theobromine were not correlated with any other metabolic perturbation and since they are present in cocoa and coffee, it is possible that FFP could contain cocoa and coffee-containing ingredients, thus explaining their higher plasma level in SA and SU groups. Considering pigs, the SA and SU diets significantly modulated few liver proteins (125 out of 2881 total identified) mostly related to lipid metabolism and cellular structure but with no metabolic interaction among them, indicating a limited impact of FFP on liver proteome. Plasma peptidomes were not significantly different among groups, although three peptides related to vascular homeostasis and anti-hypertension were exclusively identified in the SA group, suggesting a potential self-regulating response to face the higher Na content of the SA diet. To conclude, the 30% dietary inclusion of FFPs in piglet and pig diets did not lead to significant unintended effects on metabolism assessed at multiple levels, thus preserving the wellbeing of FFP-fed animals. Therefore, a wider application of FFP as feed ingredients is advisable to reduce the environmental impact of pig production, although further research is needed to strengthen all these outcomes.

Funding
Research supported by One Health Action Hub 2022-PSR 2021-GSA-Linea 6 from University of Milan

Glutamine and glucose metabolism in suckling low and normal birthweight piglets supplemented with glutamine

Q.L. Sciascia[1], D. De Leonardis[1], S. Görs[1], A. Vernunft[1], A. Eggert[1], J. Zentek[2], C.C. Metges[1,3]
[1]Research Institute for Farm Animal Biology (FBN), Dummerstorf, Germany. [2]Freie Universität Berlin, Faculty of Veterinary Medinine, Berlin, Germany. [3]University of Rostock, Faculty of Agricultural and Environmental Sciences, Rostock, Germany (metges@fbn-dummerstorf.de)

Glutamine (Gln) improved intestinal integrity and structure, and immune and antioxidative status in post-weaning piglets (Ji et al. 2019). Piglets are especially vulnerable in early phases of life and it was hypothesized that Gln supply with milk is not adequate for suckling piglets. Only few reports are available on the effects of Gln supplementation in neonatal suckling piglets. Almost nothing is known on Gln and glucose (Glc) metabolism in low birthweight (LBW) piglets which are disadvantaged in terms of later growth and organ maturation. We examined the effect of Gln supplementation on the oxidation and plasma rate of appearance of Gln and Glc, and the contribution of Gln carbon for Glc synthesis in LBW vs. normal birthweight (NBW) piglets. Neonatal male German Landrace piglets with a birthweight of 1.04 ± 0.02 kg in LBW, and 1.55 ± 0.02 kg in NBW were assigned to a two-by-two factorial design (birthweight/supplement). Glutamine (1 g/kg bodyweight /d) suspended in water was given to half of the LBW and NBW piglets, whereas the other half received water (W) until age day (d) 15 (LBW-Gln, NBW-Gln: n=12; LBW-W, NBW-W: n=11). At 12 d, a jugular vein catheter was fitted. Piglets were subjected to three oral tests: on 14 d with $^{13}C_5$-Gln/Gln, on 15 d with lactulose/mannitol and on 15 d with $^{13}C_6$-Glc/Glc and xylose. Frequent blood samples were taken over five hours. We analysed ^{13}C enrichments of tracers in plasma and CO_2 in red blood cells (RBC) to determine plasma Gln and Glc rate of appearance (Ra), ^{13}C recovery (^{13}C REC) in RBC CO_2 from $^{13}C_5$-Gln and $^{13}C_6$-Glc as a proxy for oxidation and $^{13}C_5$-Gln conversion to $^{13}C_3$-Glc to assess gluconeogenesis, as well as the plasma lactulose, mannitol, xylose and urea. The LBW piglets supplemented with Gln had a lower bodyweight than those of the control group ($P<0.05$) but Gln did not affect daily weight gain, abdominal girth, crown-rump length, and body mass index which was higher in NBW than LBW piglets ($P<0.01$). Plasma $^{13}C_6$-Glc Ra did not differ among groups and was higher than that of $^{13}C_5$-Gln Ra in all groups ($P<0.01$), whereas the $^{13}C_5$-Gln to $^{13}C_3$-Glc conversion did not differ among groups ($P>0.1$). The ^{13}C REC values of Gln and Glc were higher in LBW than in NBW piglets ($P<0.01$), while the ^{13}C REC of Gln was higher than that of Glc regardless of birthweight and supplementation ($P=0.005$). The xylose and mannitol absorption tests suggested a greater intestinal absorption capacity/area in LBW piglets ($P<0.05$); plasma urea was higher in Gln supplemented groups ($P<0.05$). In summary, Gln supplementation did not have a consistent effect on the measured parameters, but LBW and NBW piglets differ in their Gln and Glc metabolism.

Reference

Ji, F.J., Wang, L.X., Yang, H.S., Hu, A., Yin, Y.L., 2019. Animal 13, 2727–2735.
https://doi.org/10.1017/S1751731119001800

Funding

European Union’s Horizon 2020 research and innovation programme; Marie Skłodowska-Curie Innovative Training Network; grant agreement No 955374.

The effect of glutamine supplementation on low birthweight suckling pig growth, organ mass and glutamine metabolism.

Q.L. Sciascia[1], D. De Leonardis[1], S. Görs[1], J. Zentek[2], C.C. Metges[1]
[1]Research Institute for Farm Animal Biology (FBN), Dummerstorf, Germany. [2]Freie Universität Berlin, Berlin, Germany (metges@fbn-dummerstorf.de)

Low birthweight (L) pigs have impaired growth and small intestine (SI) development compared to normal birthweight (N) littermates, and glutamine (Gln) supplementation improves these impairments in L suckling pigs. However, it is not known if Gln supplementation alters SI mass, amino acid (AA) metabolism or the metabolic fate of Gln. Therefore, this study investigated piglet growth, SI mass, duodenal AA and Gln metabolism in Gln supplemented L suckling pigs. At birth (1 day (d) of age), 50 male L (0.8–1.2 kg) and N (1.5–1.9 kg) littermates were selected, and supplemented (2 to 19 d) with Gln (1 g/kg bodyweight/d: L-Gln/N-Gln) or water (L-W/N-W). Bodyweight, length and width were recorded and average daily gain (ADG), ponderal (PI) and bodymass index (BMI) calculated. Prior (1.5 h) to slaughter (20 d), pigs were given an oral bolus of 10 mg/kg bodyweight $^{13}C_5$-Gln to trace Gln metabolism. Blood samples were collected at birth (0, 4 h) to measure plasma metabolites and free AA concentrations and at slaughter to measure red blood cell glutathione concentrations and plasma enrichment of $^{13}C_5$-Gln, $^{13}C_3$-glucose (Glc) and $^{13}C_2$-Glc. Small intestine length and empty weight was measured and duodenum tissue sub-sampled to measure total AA and $^{13}C_5$-Gln enrichment. Data was analysed using the GLIMMIX procedure of SAS. From 17 to 20 d, N-Gln were heavier than N-W. The ADG was higher in N-Gln than N-W and in N than L ($P<0.05$). From birth to 20 d, L pigs were thinner, shorter and lighter ($P<0.05$) than N, whilst BMI was lower at 2, 8 and 20 d in L-Gln than N-Gln, and at 8, 15 and 20 d in L-W than N-W ($P<0.05$). At 8 d, L-Gln were longer, at 15 d had a higher BMI, and at 2 and 15 d their PI was higher than L-W ($P<0.05$). At birth, the concentrations of five plasma AA, citrulline, β-alanine, albumin and fructose were lower and carnosine, 1-methylhistidine, lactate and inositol were higher in L than N ($P<0.05$). At 4 h post-birth, levels of twelve plasma FAA, essential AA, ornithine and inositol were higher and glutamate, carnosine, albumin, glucose and total protein were lower in L than N ($P<0.05$). At slaughter, duodenum, caecum and SI were heavier and the SI longer in N than L ($P<0.05$). The stomach was heavier and duodenum longer in N-Gln than L-Gln, whilst the SI was heavier in N-Gln than N-W ($P<0.05$). Duodenal total proline concentrations were lower in N-Gln than N-W ($P=0.03$). Plasma enrichment of $^{13}C_2$-Glc was higher in L-Gln than L-W ($P=0.04$). Taken together these results show an altered glucose metabolism in L compared to N and Gln supplementation only improved suckling N pig growth and SI development.

Funding
European Union's Horizon 2020 research and innovation programme; Marie Skłodowska-Curie Innovative Training Network; grant agreement No 955374.

Impact of variation in dietary amino acid level throughout the day on the blood metabolites of pigs

A.M. Veira[1], C.A. Silva[1], L.S. Santos[2], D.A. Marçal[1], A.Z. Fraga[1], L. Hauschild[1]
[1]São Paulo State University (Unesp), Jaboticabal, Brazil. [2]Federal University of Mato Grosso do Sul, Campo Grande, Brazil (alini.mari@unesp.br)

Feeding programs in pig nutrition do not consider variations in metabolism that occur during the 24-hour-day cycle. However, nutrient metabolism changes according to the animals' circadian rhythm. Therefore, we hypothesized that adjusting diet amino acids (AA) concentration according to the circadian rhythm could improve the nutrient utilization by pigs. Thus, the objective was to evaluate the effect of adjusting the diet AA concentration according to circadian rhythm on the blood metabolites of growing pigs. Twenty-four pigs (35 ± 3.32 kg) were assigned to a randomized block design in three treatments: daily feeding program (DP), where a diet with 100% of estimated AA requirements was provided in the morning and afternoon; and two sequential feeding program (SEQ): one with low AA concentration (-40%) in the morning and high (+40% AA) in the afternoon (SEQLH), and other with high (+40%) in the morning and low (-40% AA) in the afternoon (SEQHL). Pigs were housed individually in metabolic cages. The experiment lasted 21 days and blood samples were collected on days 5, 14 and 21. On each day, blood samples were collected twice with the first collection performed after an overnight fast (pre-prandial), and the second carried out 2h30min after the first morning meal (postprandial). Quantitative determination of lactate, glucose, triglycerides, albumin, total protein, and urea were performed. Data was analyzed using a mixed model, considering the fixed effect of the feeding program, the block as a random effect, and using day five as a covariate. The adjusted means were compared by Tukey at 95% significance. The pre and postprandial glucose, triglycerides, and total protein blood concentration were not affected among the groups ($P > 0.05$). Pre and postprandial lactate concentration was higher ($P < 0.05$) for SEQHL on day 21 compared to SEQLH (25 vs 15 and 32 vs 20 mg/dL, respectively). Pigs in DP and SEQLH had higher ($P < 0.05$) concentrations of pre-prandial albumin on day 14 compared to SEQHL (1.35 and 1.27 vs 1.07 g/dL). However, on day 21, pre-prandial albumin concentration was lower ($P < 0.05$) in SEQLH compared to SEQHL (2.30 vs 2.63 g/dL). Additionally, the postprandial albumin blood concentration was not affected between the groups ($P > 0.05$). Pre and postprandial urea concentration was higher ($P < 0.05$) for SEQHL on both days compared to DP and SEQLH (Pre-prandial: Day 14 - 8.6 vs 6.7 and 7.1 mg/dL; Day 21 – 12.5 vs 9.8 and 9.2 mg/dL; Postprandial: Day 14 – 14.2 vs 10.1 and 10.2 mg/dL; Day 21 – 13.5 vs 10.1 and 8.3 mg/dL). Providing a high diet AA concentration in the morning and a low in the afternoon increased serum lactate and urea levels, suggesting that the energy and protein metabolism may have been compromised.

Funding

This research was funded by the São Paulo Research Foundation – FAPESP (grant number: 2018/15559-7). Alini Mari Veira was supported by São Paulo Research Foundation – FAPESP (grant number: 2017/18734-1) and the National Council for Scientific and Technological Development – CNPq (grant number: 151531/2024-2).

Effect of dietary amino acid to energy ratio on performance and nutrient deposition of broiler chickens fed various density diets

H. Chen, E. van der Hoeven-Hangoor, L.M. van Eck, H. Enting
Cargill Animal Nutrition and Health, Velddriel, Netherlands (Hsuan_Chen@cargill.com)

Dietary energy change is frequently applied in broiler chicken production to enhance growth rates and improve feed conversion ratios (FCR). Adjustments to dietary amino acid (AA) levels may be necessary in high energy diets to maintain nutrient intake balance for optimal utilization efficiency. This study aimed to evaluate the effect of AA to energy ratio (AA:ME) on performance, nutrient deposition and utilization efficiency in broilers fed various energy diets. A 3 (dietary energy level) x 2 (fixed or decreased AA:ME) factorial design was applied in the current study. A total of 576 chickens were randomly divided over 54 pens, with 12 chickens per pen. The study was divided into three phases: starter (day 0-10), grower (day 10-23), and finisher (day 23-35). The lowest metabolizable energy levels for each phase were 2800, 3000, and 3100 kcal/kg with an incremental increase of 150 kcal/kg by higher soy oil inclusion for the other two treatments. Dietary digestible lysine was either maintained constant at 1.25%, 1.20% and 1.10% or increased with energy with a fixed AA:ME of 4.46, 4.42 and 3.74 per feeding phase. Ideal protein profile was maintained. Average daily gain (ADG) and average daily feed intake (ADFI) were measured, and FCR was calculated. Excreta were collected on day 9, 22, and 34 for nutrient excretion. Chickens were dissected on day 10 and 35 for body chemical composition, and nutrient deposition and utilization efficiency were calculated. Data were analysed using mixed model in R. Throughout the experimental period, ADFI decreased by 2.3 g with every increase of 100 kcal in dietary energy, while overall energy intake increased linearly by 470 kcal, independent of AA:ME. A linear interaction in FCR was observed between dietary energy and AA:ME, with broilers showing a more pronounced improvement in FCR by 5 points when both dietary energy and AA increased simultaneously. Similar results were found by Aftab (2019), who concluded that modern broilers are more responsive to dietary AA density due to genetic selection. Feeding broilers with decreased AA:ME linearly reduced body protein deposition by 5% with a lower breast meat yield by 3%, resulting from lower AA intake. Additionally, fat pad yield increased from 1.15 to 1.63% in these broilers, likely due to an energy oversupply. This is consistent with the findings from Toghyani et al. (2025) that reduced dietary energy resulted in lower fat pad weight in broilers. Maintaining AA:ME while increasing dietary energy resulted in a linear reduction in fat excretion by 5% throughout the experimental period. In conclusion, maintaining dietary AA:ME when adjusting energy levels is recommended to optimize broiler efficiency and protein deposition, potentially improving sustainable poultry production with less nutrient excretion.

References

Aftab, U., 2019. World's Poultry Science Journal 75, 507–514.
https://doi.org/10.1017/S0043933919000564
Toghyani, M., MacElline, S., Selle, P.H., Liu, S.Y., 2025. Poultry Science 104, 104530.
https://doi.org/10.1016/j.psj.2024.104530

Effect of short-term adaptation with functional amino acid supplementation on immune response and protein metabolism and body composition of growing pigs raised in poor sanitary conditions

I. Franca[1], G. A. C. Valini[1], P. R. Arnaut[1], C. A. Silva[1], M. J. K. Oliveira[1], D. A. Marçal[1], A. D. B. Melo[1], J. K. Htoo[2], H. G. Brand[2], I. Andretta[3], L. Hauschild[1]
[1]Department of Animal Science, School of Agricultural and Veterinarian Sciences, São Paulo State University, Jaboticabal, Brazil. [2]Evonik Operations GmbH, Hanau, Germany. [3]Department of Animal Science, Federal University of Rio Grande do Sul, Porto Alegre, Brazil (luciano.hauschild@unesp.br)

Considering the fact that activation of the immune system alters amino acid (AA) requirements on pigs, AA-based diets strategies may attenuate the impact of sanitary challenges (SC; Rodrigues et al. 2021). The objective of this study was to evaluate the effects of extra supplementation of functional amino acids (FAA) preventively (pre-SC), curatively (during-SC), or both, on the immune response and protein metabolism and body composition of growing pigs challenged with *Salmonella* Typhimurium (ST) and poor housing conditions. Fifty-two male pigs (21.7 ± 4.6 kg) were randomly assigned to one of four treatments: 1) control AA profile diet (NRC, 2012) provided throughout the experimental period (pre-SC and during-SC), 2) FAA-enriched profile diet (FAA+; +20% Trp:Lys, Thr:Lys, and Met+Cys:Lys) supplied pre-SC (Preventive), 3) FAA+ diet supplied during-SC (Curative) and 4) FAA+ supplied pre and during-SC (Continuous).Following a 7-day pre-SC (day −7 to 0), all pigs underwent SC, which involved oral inoculation with 2×10^9 CFU of ST and manure spreading on room floors, and the room was not cleaned during the SC for 28 days. Pigs were housed in groups, and diets were delivered through precision feeders during pre-SC and SC periods. Body composition was assessed using dual-energy X-ray absorptiometry on day 0 and 28. Blood samples were collected on days 0, 7 and 28. Data were analyzed using a mixed model of SAS with initial body weight as a covariate, and least square means were compared using Tukey's test. All pigs tested positive for ST fecal shedding after inoculation. Lymphocyte count and hemoglobin concentration decreased on day 7 and 28, respectively ($P < 0.01$). Granulocyte count increased on day 7 but decreased on day 28 ($P < 0.05$). On day 7, pigs exhibited increased ($P < 0.05$) haptoglobin, ceruloplasmin, and IgA concentrations. On day 28, transferrin, α-1-acid glycoprotein, serum α-1 antitrypsin, and IgG levels were higher ($P < 0.05$) than on day 0 and 7. FAA supplementation during SC (day 0 to 28) reduced serum albumin concentration ($P < 0.01$) and increased transferrin concentrations in curative and continuous treatments compared to control group ($P < 0.05$). During SC, pigs in the curative FAA treatment had higher total blood protein concentrations ($P < 0.01$) than control pigs. Continuous FAA supplementation resulted in lowest plasma urea concentrations ($P < 0.01$) from day 0 to 28. No effects were observed for serum creatinine ($P > 0.05$). On day 28, body protein content tended to be higher in the curative group compared to preventive group (6.4 vs. 5.8 kg, $P = 0.07$), with improved nitrogen utilization efficiency compared to the control (57.4% vs. 43.3%, $P < 0.05$). In conclusion, curative FAA supplementation during a chronic SC positively modulated immune response and protein metabolism and body composition in growing pigs.

References

Rodrigues, L.A., Wellington, M.O., González Vega, J.C., Htoo, J.K., Van Kessel, A.G., Columbus, D.A., 2021. Journal of Animal Science 99, skab035. https://doi.org/10.1093/jas/skab035
NRC - National Research Council. 2012. The National Academies Press. https://doi.org/10.17226/13298

Funding

This work was supported by the São Paulo Research Foundation (2018/15559-7, 2019/10843-1, 2020/15797-5 and 2023/08305-7) Brazilian National Council for Scientific and Technological Development (CNPq, grants number 308779/2023-1, 408287/2021-7 and 442675/2023-2) and Evonik Operations GmbH, and Seara Alimentos - JBS Foods Brazil.

Impact of fasting and re-feeding on metabolic responses in individually precision-fed and group phase-fed pigs

A. Remus[1], C. Pomar[1], L. Hauschild[2], P.R. Arnaut[2], G.A.C. Valini[2]
[1]Agriculture and Agri-Food Canada, Sherbrooke, Canada. [2]Sao Paulo State University (Unesp), Jaboticabal, Brazil (aline.remus@agr.gc.ca)

The Individual Precision Feeding (IPF) system is a potential tool for improving nutrient utilization efficiency in pigs (Remus et al., 2020). However, it is unclear whether this increased efficiency results solely from reduced nutrient provisions or if IPF modulates metabolic pathways due to constant nutrient supply adjustments. While IPF has been associated with reduced growth performance under non-homeostatic conditions (Arnaut et al., 2023), its effects on metabolism during stress in both Group Phase Feeding (GPF) and IPF pigs remain poorly understood. This study compared the metabolic response of pigs under GPF and IPF during a 24-hour fasting and refeeding protocol. Sixty pigs (34 ± 1.16 kg BW) were assigned to either GPF or IPF for a 21-day growth phase. On day 24, ten pigs per treatment were moved to individual pens and fed at 90% of their previous feed intake, with lysine levels adjusted to 90% of GPF diets and 90% of individual requirements for IPF pigs. After a 4-day adaptation period with twice-daily meals, blood samples were collected at four-time points: (1) after a 12-hour fast on day 28 (baseline), (2) 2 hours post-feeding (300 g meal), (3) after 24 hours fasting, and (4) 48-hour post-protocol start, with pigs refeed ad libitum for 12 hours before fasting again to capture post-refeeding metabolite changes. Metabolomic data were analyzed in MetaboAnalyst using fold-change analysis and pathway enrichment (Sus scrofa KEGG) to compare metabolic profiles between feeding systems. Fold-change analysis showed no differential metabolites on day 1 but identified several metabolites elevated in IPF compared to GPF on day 28 (FDR < 0.05), including aspartic acid, alanine, pipecolic acid, glutamine, and oxoproline. Minor differences were observed at fed conditions, with PC AA C42:5 increased in IPF (FDR < 0.05) and no differences after refeeding. The top 15 metabolites with temporal changes between IPF and GPF included aspartic acid, PC AA C42:5, 5-oxoproline, pipecolic acid, N-acetyl-alanine, alanine, PC AA C42:6, N-acetyl-serine, TG(16:0_38:1), urea, histidine, glutamine, PC AA C40:3, asymmetric dimethylarginine, and beta-alanine. Metabolomic analysis revealed significant pathway enrichments in IPF pigs during fasting, including Pantothenate and CoA Biosynthesis (P = 0.02), beta-Alanine Metabolism (P = 0.02), and Histidine Metabolism (P = 0.04), suggesting enhanced energy production, amino acid metabolism, and cellular protection mechanisms. Increased levels of histidine and beta-alanine during fasting may indicate that the metabolism is adapting to buffer oxidative stress and protect cells from damage in IPF pigs. This shift highlights the metabolic adaptation to stress during fasting. By activating these pathways, IPF pigs may efficiently mobilize stored energy during fasting, optimizing energy use to support physiological processes under nutrient-limited conditions. This suggests that IPF enhances nutrient utilization and adapts the pig's metabolism to maximize energy availability when food intake is restricted.

References

Remus, A., del Castillo, J.R.E., Pomar, C., 2020. Animal 14, 2032–2041. https://doi.org/10.1017/S1751731120000798
Arnaut, L., Ribeiro, M.L., Millet, S., van den Borne, J.J.G.C., Pomar, C., 2023. Journal of Animal Science 101, 133–134. https://doi.org/10.1093/jas/skad281.163

Funding

This study was supported by AAFC and Swine Cluster 4.

Impact of soybean oil on net energy and nitrogen balance in group-housed pigs allowed ad libitum access to feed

S.A. Lee, H.H. Stein
University of Illinois, Urbana, USA (sualee2@illinois.edu)

An experiment was conducted to test the hypothesis that increased soybean oil in diets for group-housed growing pigs will increase net energy (NE) and nitrogen balance. Six diets contained corn and soybean meal and increasing inclusion rates of soybean oil (i.e., 0, 1.5, 3.0, 4.5, 6.0, and 7.5%). Ratios between digestible amino acids and metabolizable energy (ME) in all diets were equal among diets. A total of 48 pigs (initial weight: 41.98 ± 3.32 kg) were used in two blocks of 24 pigs. The 24 pigs in each block were assigned to 6 calorimeter chambers with 4 pigs per chamber. Chambers in each block were allotted to a 6 × 3 incomplete Latin square design with 6 diets and 3 periods. Therefore, each block provided 3 replicates per diet for a total of 6 replicates per diet from the 2 blocks. Pigs had ad libitum access to feed and water. Diets were fed for 13 days in each period. After a 7-day adaptation period, O_2 consumption and CO_2 and CH_4 productions were measured for 6 days to calculate total heat production. Fecal and urine samples were quantitatively collected for 6 days and analyzed for gross energy (GE) and nitrogen. The statistical model included diet as fixed effect and group, period, and chamber as random effects. Contrast coefficients were used to determine the linear and quadratic effects of increasing soybean oil in diets. Results indicated that the apparent total tract digestibility (ATTD) of GE and dry matter (DM) decreased and then increased (quadratic; $P < 0.05$) by increasing soybean oil in diets although the absolute differences were very small (Table 1). Concentrations of digestible energy (quadratic; $P < 0.013$), ME (linear; $P < 0.001$), and NE (linear; $P < 0.001$) in diets increased by increasing dietary soybean oil. The respiratory quotient tended to be reduced (linear; $P = 0.059$) with increasing dietary fat. The ATTD of nitrogen decreased and then increased (quadratic; $P = 0.013$), but retention of nitrogen (% of intake and % of absorbed) increased (linear; $P < 0.05$) as dietary soybean oil increased. In conclusion, increasing dietary soybean oil increased energy digestibility and concentrations of NE in diets, and decreased the ratio between produced carbon dioxide and consumed oxygen. The linear increase in nitrogen retention (% of intake and % of absorbed) indicated that more body protein was synthesized as increased dietary soybean oil made more energy available for the pigs.

Funding

The financial support from the National Pork Board, Des Moines, IA, USA, is appreciated.

Energy expenditure and thermal variation in response to lipopolysaccharide challenge in broilers

N. Sakomura[1], R. Camargos[1], N. Consolo[2], R. Riveros[1], M. Macari[1]
[1]UNESP, Jaboticabal, Brazil. [2]USP, Pirassununga, Brazil (nilva.sakomura@unesp.br)

Health challenges in broiler chickens cause imbalances in metabolic, physiological, and immunological functions, directly impacting production and performance. This study aimed to elucidate the relationship between body temperature and energy metabolism in birds subjected to two degrees of lipopolysaccharide (LPS) challenge, assessing energy expenditure and exploring related metabolites. A total of 21 one day of age male Cobb 500 broilers were raised in cages until 18d. At 19d, they were assigned to three treatments: Control non-challenged birds (CON), Challenged birds with 10 µg Escherichia coli LPS/kg BW (10LPS), and Challenged birds with 100 µg/kg BW (100LPS). At 21d, the corresponding LPS dose was injected via intra-muscular (IM) (1ml solution/kg BW), and the treatment CON birds received saline. BW, gas exchange, and core body temperature were recorded for 23h. Feed was removed to measure fasting heat production (FHP). At 22 d, blood and liver samples were collected for metabolomic analysis. There was no difference on BW among treatments (P>0.05); however, the 100LPS treatment showed lower heat production (HP) (P<0.05), but with no difference compared to 10LPS treatment (P>0.05). The respiratory quotient (RQ) and body temperature were higher in 10LPS (P<0.05) when compared CON and 100LPS. The 10LPS group exhibited metabolic changes compared to the CON group, and the 100LPS group presented hypothermia and lower RQ. Additionally, was showing an increasing 1°C body temperature 0.6 h after IM-injection that resulted in an increasing on the energy expenditure in 30 kcal/kg$^{0.75}$ in birds challenge with 100LPS, result same than reported by Amaral-Silva et al, (2021). In other hand, the plateau FHP, that is the maintenance energy requirement was the same in all treatments obtaining 114 kcal/kg$^{0.75}$ and an RQ of 0.74 that is in accordance of reported in the literature (Noblet et al., 2015). A total of 50 and 51 metabolites were identified and quantified in the 1H-NMR spectra of serum and liver, respectively, including amino acids, organic acids, nucleic acids, vitamins, cellular antioxidants, and others. Comprehensive analysis of the metabolomic data showed that 17 and 27 pathways were significantly difference between treatments for serum and liver, respectively, emphasizing their use in attempting to control immunological challenge and minimize damage. By understanding how different degrees of LPS challenge affect energy metabolism, body temperature, and specific metabolomic pathways, nutrient supplied can be considered to better support energy metabolism and immune function under health challenge conditions. Additionally, the identified metabolic markers can be used to more accurately monitor the health and immune status of broilers, allowing for earlier interventions and more targeted treatments.

References

Amaral Silva, L., Gargaglioni, L.H., Steiner, A.A., Oliveira, M.T., Bicego, K.C., 2021. Journal of Physiology 11, 2969–2986. https://doi.org/10.1113/JP281385

Noblet, J., Dubois, S., Lasnier, J., Warpechowski, M., Dimon, P., Carré, B., van Milgen, J., Labussière, E., 2015. Animal 9, 1138–1144. https://doi.org/10.1017/S1751731115000403

Funding

This study was financed by the São Paulo Research Foundation (FAPESP), Brazil. Process Number 2019/26575–6.

The number of daily meals for sows has only marginal influence on plasma metabolites and the gut microbiome

K.K. Lyderik[1], R. Dhakal[1], C. Marcussen[2], T. Thymann[1], S.M. Offersen[1], A.V. Strathe[1]
[1]Department of Veterinary and Animal Sciences, University of Copenhagen, Frederiksberg, Denmark. [2]Department of Veterinary Clinical Sciences, University of Copenhagen, Frederiksberg, Denmark (avst@sund.ku.dk)

The aim of this study was to test the effect of feeding 1,2 or 3 daily meals on gut health and blood metabolites in sows. Five empty sows (Yorkshire x Landrace) were fitted with T-cannulas in the distal ileum (Stein et al., 1998) and an ear vein cathether. Sows were allowed 7 days for postsurgical adaptation before experimental feeding was initiated. Sows were fed the same daily amount of feed (2.15 kg/day, 25.4 MJ ME/day), given as either one daily ration (2.15 kg), or divided into two (1.075 kg) or three meals (0.717 kg) per day. The effect of daily meals was examined in a cross-over design, where all sows were allotted to all dietary treatments. For three sows, the experiment was repeated. Each treatment period lasted seven days, where the first five days allowed for the adaptation to a new treatment. On day 5, fecal samples were collected, and blood sampling was performed hourly for 24 hours (n=8). Samples of ileal digesta were collected on days 6 and 7 (n= 21). Short-chain fatty acids (SCFA) and microbiota (16s rRNA) of feces and ileal digesta were analyzed, and serum was analyzed for glucose, insulin, lactate and SCFA. Sow was regarded as the experimental unit, and the statistical model included the number of daily meals as a fixed effect and sow and period as random effects. In feces the concentrations of SCFA were unaffected by dietary treatment. Concentrations of propionic acid and isovaleric acid were lower in sows fed 1 meal compared to sows fed 3 meals ($P < 0.05$). Concentrations of propionic acid and isovaleric acid were higher in feces compared to ileal digesta ($P < 0.001$), whereas concentrations of acetic acid and n-butyric acid were similar. There was a tendency for higher alpha diversity of fecal microbiota in sows fed 1 meal compared to sows fed 2 meals (Shannon index: 8.4 vs. 7.8 and 6.7; $P=0.08$). Alpha diversity of microbiota was higher in feces compared to ileal digesta (Shannon index: 7.5 vs. 5.8; $P<0.01$). The 24 h average concentrations of glucose, lactate and SCFA were similar between groups, whereas there was a tendency for a higher average insulin concentration in sows fed 1 meal compared to 2 or 3 meals (4.1 vs. 1.3 and 1.1 mmol/L; $P=0.07$). In conclusion, the preliminary results of the study showed that sows in all groups effectively regulate their blood glucose concentration, resulting in higher concentrations of blood insulin of sows fed 1 meal compared to 2 and 3 meals. Higher concentrations of some SCFA in ileal digesta of sows fed 3 small meals compared to 1 large meal could indicate that the very small meals increase fermentation in the distal ileum.

Reference

Stein, H.H., Shipley, C.F., Easter, R.A., 1998. Journal of Animal Science 76, 1433–1436.
https://doi.org/10.2527/1998.7651433x

Funding

The Danish Pig Levy Foundation and Norma and Frode S. Jacobsens Fund

Finishing pigs fed low protein diets require lower methionine plus cysteine levels than expected

S. Goethals[1], P. Bikker[2], A. Jansman[2], S. Millet[1]
[1]ILVO, Merelbeke-Melle, Belgium. [2]WUR, Wageningen, Netherlands (Sam.Millet@ilvo.vlaanderen.be)

Supplementing amino acids to low protein diets is an effective strategy to reduce nitrogen excretion without compromising pig performance. Therefore, accurate determination of the requirements of limiting amino acids in such diets is crucial. Sulfur amino acids (SAA; methionine (Met) and cysteine (Cys)) are considered the second or third limiting amino acids in pigs and are typically expressed relative to lysine (Lys). A dose-response study was conducted using 240 finishing pigs (80-115 kg) to evaluate the effects of standardized ileal digestible (SID) SAA to Lys ratio on growth performance, carcass characteristics and serum urea level, a marker for nitrogen efficiency. A basal diet (6.3 g SID Lys /kg and 114 g crude protein/kg) was supplemented with graded levels of crystalline methionine (L-Met), resulting in dietary SID SAA:Lys ratios of 47.5%, 52.5%, 57.5%, 62.5%, 67.5% or 72.5% for Treatment 1-6. Pens, with five pigs each, were allocated to one of six diets with eight pens per treatment. We hypothesized that the SAA requirement could be estimated using a linear-plateau model, identifying the minimum SAA level beyond which growth performance would not be improved. Preliminary results indicated that Met supplementation did not result in the expected linear-plateau response in growth performance parameters, in contrast to a similar dose-response study (Ma et al, 2016), which indicated a requirement of 58% SID SAA:Lys based on feed conversion ratio as response criterion. In the present study, daily gain (g/d) was 941^{b}, 1069^{a}, 946^{b}, 992^{ab}, 1003^{ab}, 984^{ab} (SEM=45g/d; *P*=0.015); daily feed intake (g/d) was 2994^{a}, 3118^{a}, 2898^{a}, 3010^{a}, 3015^{a}, 2973^{a} (SEM=81g/d; *P*=0.268) and G:F (kg/kg) was 0.31^{b}, 0.34^{a}, 0.33^{ab}, 0.33^{ab}, 0.33^{ab}, 0.33^{ab} (SEM=0.01kg/kg; *P*=0.092) for Treatment 1-6, respectively. Post-hoc tests indicated higher daily gain at 52.5% SID SAA:Lys compared to 47.5% and 57.5%, and a higher G:F at 52.5% compared to 47.5%. The reason for this apparent effect of the 52.5% SID SAA:Lys diet remains unclear. Serum urea levels, however, decreased slightly but consistently with increasing Met supplementation (*P*=0.018), suggesting improved nitrogen efficiency when increasing the SAA level. Mean serum urea levels (mg/dL) were 16.9^{a}, 16.5^{a}, 16.4^{a}, 14.3^{a}, 13.8^{a}, 13.7^{a} (SEM=1.2mg/dL; *P*=0.247) for Treatment 1-6, respectively. Carcass traits such as dressing yield, lean meat percentage, backfat thickness and loin muscle depth were not significantly affected by SAA inclusion level. In conclusion, increasing SID SAA:Lys ratio did not result in the expected linear-plateau response in growth performance. Instead, daily gain and feed efficiency were highest at 52.5% SID SAA:Lys, which is lower than previously reported requirement estimates. A higher ratio did not improve performance or carcass quality. However, serum urea decreased with increasing SAA:Lys ratio, suggesting improved nitrogen efficiency at higher SAA levels.

Reference

Ma, W., Zhu, J., Zeng, X., Liu, X., Thacker, P., Qiao, S., 2016. Animal Science Journal 87, 76–83.
https://doi.org/10.1111/asj.12398

Funding

This study was funded by the VLAIO COOCK+ project OPTEVAR (HBC.2023.0468).

Functional amino acids attenuate the sanitary challenge impact on maintenance requirements and improve pigs' robustness to immune system stimulation

I. França[1], G.A.C. Valini[1], P.R. Arnaut[1], C. A. Silva[1], M.J.K. de Oliveira[1], D.A. Marçal[1], A.D.B. Melo[1], J.K. Htoo[2], H.G. Brand[3], I. Andretta[4], L. Hauschild[1]
[1]Department of Animal Science, School of Agricultural and Veterinarian Sciences, São Paulo State University, Jaboticabal, Brazil. [2]Evonik Operations GmbH, Hanau-Wolfgang, Germany. [3]Evonik Brasil Ltda, São Paulo, Brazil. [4]Department of Animal Science, Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil (ismael.franca@unesp.br)

During immune system stimulation (ISS), pig growth is compromised as nutrients are redirected to support immune functions (Pastorelli et al., 2012). Functional amino acids (FAA), including threonine, tryptophan, and methionine, may mitigate these effects by modulating inflammatory response, preserving gut integrity, and protecting against oxidative stress. This study evaluated the effects of supplementation with threonine, tryptophan, and methionine, provided either preventively (pre-ISS), curatively (during-ISS), or continuously (pre and during ISS) on pig performance and maintenance requirement. Fifty-two male pigs (21.7±4.6 kg) were randomly assigned to one of four treatments: 1) control AA profile diet (NRC, 2012) provided throughout the experimental period (pre-ISS and during-ISS), 2) FAA-enriched profile diet (FAA+; +20% Trp:Lys, Thr:Lys, and Met+Cys:Lys) supplied pre-ISS (Preventive), 3) FAA+ diet supplied during-ISS (Curative) and 4) FAA+ supplied pre and during-ISS (Continuous). The preventive group received the control diet during the ISS. Following a 7-day pre-ISS (day −7 to 0), all pigs were inoculated with *Salmonella* Typhimurium (2×10^9 CFU/mL) on day 0. Fresh manure from a commercial pig farm was spread on the pen floor, and the room remained unclean during the ISS (0 to 7 ISS day). Body weight was recorded on days −7, 0, and 7, and blood samples were collected on days 0 and 7. Average daily feed intake (ADFI) was recorded using precision feeders. Each pig was considered the experimental unit. To assess the relationship between ADFI and average daily gain (ADG) percentage changes (Δ) in both variables were calculated based on individual information of pre-ISS (day -7 to 0) and after ISS (day 0 to 7). These relative data were used to derive equations estimating the relationship between the change in ADG (ΔADG) and in ADFI (ΔADFI) using the REG procedure of SAS. At day 7, pigs exhibited increased ($P<0.05$) concentrations of haptoglobin, ceruloplasmin, and IgA, indicating an acute immune response. During the ISS, ADG reductions of 33, 39, 50, and 77% were observed in the preventive, continuous, curative, and control treatments, respectively. A quadratic regression model described the relationship between ΔADFI and ΔADG, with negative intercept values in all treatments, indicating that part of the ΔADG varied independently of ADFI (−38, −17, −30, and −12% for control, preventive, curative, and continuous groups, respectively). This suggests greater maintenance requirement cost in the control and curative groups. The reductions in ADG explained by impaired feed efficiency were 51, 49, and 68% for the control, preventive, and curative treatments, respectively, whereas in the continuous FAA supplementation group, this value was 40%. Functional AA supplementation, particularly when provided continuously, attenuated the growth decline under an immune challenge by reducing maintenance requirement costs and improving feed efficiency.

Reference

Pastorelli, H., Montagne, L., van Milgen, J., 2012. Animal 6, 952–961.
https://doi.org/10.1017/S175173111100228X

Funding

This work was supported by the São Paulo Research Foundation (2018/15559-7, 2019/10843-1, 2020/15797-5 and 2023/08305-7), and Brazilian National Council for Scientific and Technological.

Impact of functional amino acids supplementation on non-targeted metabolomics profiling of serum in pigs under a health challenge

I. França[1], A. M. Veira[1], D. A. Marçal[1], I. Andretta[2], A. M. L. Ribeiro[2], L. S. dos Santos[3], C. R. Lehnen[4], C. M. Nyachoti[5], L. Hauschild[1]

[1]School of Agricultural and Veterinarian Sciences, São Paulo State University, Jaboticabal, Brazil. [2]Faculty of Agricultural and Life Sciences, Federal University of Rio Grande do Sul, Porto Alegre, Brazil. [3]Faculty of Veterinary Medicine and Animal Science, Federal University of Mato Grosso do Sul, Campo Grande, Brazil. [4]State University of Ponta Grossa, Ponta Grossa, Brazil. [5]Faculty of Agricultural and Food Sciences, University of Manitoba, Winnipeg, Canada (ismael.franca@unesp.br)

Functional amino acids (FAA) may support immune responses by modulating inflammation, preserving gut integrity, and reducing oxidative stress during immune system stimulation (ISS). Metabolomics offers a comprehensive approach to identifying metabolic changes and pathways (Goldansaz et al., 2017). This study evaluated the effects of FAA-blend supplementation before and/or during ISS on blood metabolites and metabolic pathways in growing pigs. Forty piglets (6.6 ± 0.7 kg BW) were distributed to one of two diets (n=20): control diet (CN; NRC, 2012) or a diet supplemented with FAA (FAA+; Thr:Lys, Trp:Lys, and Met+Cys:Lys at 120% of CN levels). The nursery phase lasted seven weeks. At the beginning of the growth phase (28.8 ± 2.3 kg BW), pigs were randomly assigned to one of four experimental strategies considering the diets provided during the pre-ISS (nursery): pigs fed CN diet in the nursery either continued on CN (Control; n=10) or switched to FAA+ (Curative; n=10) during the ISS phase. Similarly, pigs fed FAA+ during nursery phase were either switched to CN (Preventive; n=10) or continued on FAA+ (Continuous; n=10) during the ISS. The ISS involved exposing pigs to batch mixing, poor housing conditions, and oral inoculation with Salmonella Typhimurium. The growth phase lasted four weeks. Blood samples were collected at the end of the challenge period after six hours of fasting. Plasma was analysed using GC-MS and data were processed with ChromaTOF and the NIST library was used for metabolite identification. Statistical analyses were performed using MetaboAnalyst 6.0. A total of 111 metabolites were identified, mainly involved in protein (n = 26), energy (n = 17), lipid (n = 13), and immune metabolism (n = 11). Partial least square discriminant analysis (PLS-DA) revealed three distinct clusters, where the Control and Continuous-FAA+ showed unique profiles, while Preventive-FAA+ and Curative-FAA+ exhibited similarities. Variable importance projection indicated that the 10 metabolites most contributed to group separation were primarily associated with energy metabolism and oxidative stress regulation. ANOVA identified 59 differentially expressed metabolites, with Preventive and Curative groups sharing similar metabolic responses. Heatmap analysis confirmed a reduced abundance of several metabolites in the Control, reinforcing its distinct metabolic status. Enrichment and pathway analyses (KEGG library Sus scrofa) revealed significant treatment-induced alterations in the metabolic pathways of alanine, aspartate, and glutamate; taurine and hypotaurine; starch and sucrose; and phenylalanine, tyrosine, and tryptophan, suggesting changes in amino acid utilization, energy regulation, and oxidative stress. In summary, the Control exhibited a distinct metabolic profile, whereas Preventive-FAA+ and Curative-FAA+ shared similarities, and the Continuous-FAA+ displayed a unique metabolic adaptation, which may indicate long-term physiological adjustments. These findings indicate FAA supplementation may play a key role in supporting metabolic resilience under ISS and may improve stress adaptation in pigs.

Reference

Goldansaz, S.A., Zhang, Y.M., Guerzoni, M., 2017. PLOS ONE 12, e0177675. https://doi.org/10.1371/journal.pone.0177675

Funding

São Paulo Research Foundation (2018/15559-7, 2019/10843-1, 2020/15797-5 and 2023/08305-7), Brazilian National Council for Scientific and Technological Development (CNPq, grants number 308779/2023-1, 408287/2021-7 and 442675/2023-2) and Evonik Operations GmbH, and Seara Alimentos - JBS Foods Brazil.

Evaluation of the standardised ileal digestible lysine requirement of 6 to 9 kg weaner pigs

S. Laird, L. Blavi, L. Sobrevia, S. Tibble, A. Koppenol
AB Neo, Fraga, Spain (steven.laird@ab-neo.com)

As the first limiting amino acid for muscle protein accretion in practical pig diets, accurate estimations of lysine (Lys) requirements are crucial for maximising performance while minimising nitrogen excretion. Although lysine requirement estimations are available across various stages of growth for the pig, regular revaluation is important due to ongoing advancements in the genetic capacity for growth and significant changes in diet formulation. The purpose of this study was to estimate the standardised ileal digestible (SID) lysine requirements of newly weaned pigs from 6 to 9 kg BW. A total of 2,400 DanBred x Pietrain pigs were weaned onto the 12-day feeding trial at ~24 days of age with an initial BW of 6.28 ± 0.88 kg. Pigs were allotted into single-sex pens of 25 pigs/pen based on body weight. Pens were randomly allocated to one of eight dietary treatments according to a randomised complete block design with 12 replicates per treatment. The eight treatments were formulated to contain 1.05, 1.12, 1.19, 1.26, 1.33, 1.40, 1.47, 1.54 SID Lys (%), with intermediate doses created through mixing of the two extremes. Diets were isoenergetic (10.40 MJ NE/kg) and formulated to meet or exceed the requirement for all nutrients except Lys. The increase in SID Lys was achieved through the inclusion of both crystalline lysine and proteinaceous ingredients and a reduction in wheat. Pigs were weighed at the pen level at the start and end of the trial, along with feed refusals for the determination of overall average daily gain (ADG), average daily feed intake (ADFI) and feed conversion ratio (FCR). Faecal consistency scores were recorded daily at the pen level throughout the study. Orthogonal polynomial contrasts were used to test for linear and quadratic effects of increasing SID Lys (JMP Pro®, Version 18.0). For the overall treatment period, increasing SID Lys linearly improved ($P < 0.001$) ADG and FCR ($P < 0.001$), without affecting ADFI ($P > 0.05$). Similarly, the incremental increase in dietary SID Lys linearly increased faecal consistency scores (i.e. wetter $P < 0.001$), which can likely be explained by the concomitant increase in CP associated with increasing SID Lys. As there was no sign of a flattening of the curve within the range of SID Lys levels tested (quadratic $P > 0.05$) for any aspect of growth performance, these results suggest that the dietary SID Lys requirement is greater than the second highest tested level of 1.47% (1.41 g SID Lys/MJ NE). In summary, weaner pigs of 6 to 9 kg BW respond to increasing dietary SID Lys from 1.05 to 1.54% in a linear manner. However, the improvements in performance in this study were met with increasingly looser faeces, highlighting the trade-off between performance and post-weaning diarrhoea risk.

Impact of dietary isoflavones in standard chow on reproductive development and hepatic fat accumulation in female mice with different metabolic phenotypes

Z. Meyer[1], A. Lubs[1], D. Ohde[1], C. Walz[1], J. Schoen[2,3,1], D. Dannenberger[1], H. S. Willenberg[4], A. Hoeflich[1], J. Brenmoehl[1]

[1]Research Institute for Farm Animal Biology (FBN), Dummerstorf, Germany. [2]Leibniz Institute for Zoo and Wildlife Research IZW, Berlin, Germany. [3]Technische Universität Berlin, Berlin, Germany. [4]University Medicine Rostock, Rostock, Germany (brenmoehl@fbn-dummerstorf.de)

Plant-based animal feed often contains soy and red clover in varying proportions, resulting in different levels of isoflavones (IFs). Based on their structural similarity to 17β-estradiol, IF metabolites can exhibit estrogenic or anti-estrogenic effects, influencing body composition, metabolism, and fertility. We investigated the impact of three standard diets with varying IF concentrations (diet A: 0.6, S: 0.4, SPA: 0 mg IF equivalents/g diet) on three mouse lines derived from the same strain but differing in body weight and metabolism due to long-term selection, including obese (DU6), metabolically flexible (DUhTP), and non-selected control (DUC). We determined dietary IF effects on reproductive development, performance, body composition, and hepatic fat deposition. IF-containing feed led to pharmacologically relevant IF plasma concentrations (>1.9 µmol/L) in all lines. No significant effects of the diet on body or organ weight were observed in any of the mouse lines. However, the onset of puberty was accelerated in all animals in an IF dose-dependent manner, albeit to varying degrees. Compared to the diet without IFs, the accelerated effect in DUhTP mice with higher metabolic activity, typically characterized by delayed puberty, was more pronounced (A: - 9 days, S: -4 days) than in DU6 mice (A, S: -2 days), which already have an earlier onset of puberty. A dose-dependent effect of IFs on gestation length was also observed in all lines ($p<0.05$). However, increasing IF concentrations in the diet led to prolonged gestation in DUC mice and a shortening in DU6 and DUhTP mice. In obese DU6 mice, pregnancy rates decreased to 58% with higher IF exposure (diet A) in contrast to DUC (83%) or DUhTP mice (94%). With increasing IF content in the feed ($0 < 0.4 < 0.6$ mg IF/g diet), the number of lipid droplets (LD) immunohistochemically detected in the liver of the DUC mice decreased ($p<0.05$). Significant or dose-dependent differences in hepatic fatty acid concentrations were not observed. In contrast, fewer (compared to A: -47%, S: -34%) but larger LDs (compared to A: 4.4-fold, S: 2.2-fold) were detected in the liver of DU6 mice when no IF concentrations were present in the diet compared to feed with IFs ($p<0.01$). At the same time, significantly higher concentrations of fatty acids (FA) and a higher proportion of monounsaturated FA were observed in the livers of the obese DU6 mice when the diet did not contain IFs ($p<0.001$). In DUhTP mice with higher metabolic flexibility, the different diets did not affect hepatic FA accumulation. Our findings indicate that varying IF concentrations in standard feeds, caused by ingredient composition, exhibit estrogenic or anti-estrogenic effects depending on the metabolic status of the analyzed mouse line. Feed composition and metabolic status interaction may also impact livestock and requires further investigation.

Influence of a protein restricted diet on organ weight and performance in male dual-purpose and male layer chickens

A. M. Rettig, A. Kaiser, F. Lange, F. Böttcher, G. Trei, E. Saliu
Hochschule für nachhaltige Entwicklung, Eberswalde, Germany (alexander.rettig@hnee.de)

Consumer demands and political pressure to raise male dual-purpose chickens (DPC) and male layer chickens (MLC) is increasing. However, scientific knowledge on nutrient requirements for these chickens is limited. This study aimed to analyze the effect of protein-reduced feeding on the performance and organ development of DPC and MLC. A total of 320 roosters (160 ÖTZ Coffee (DPC), 160 ISA Warren (MLC)), were raised from week 8-16 (EU Reg. 2018/848). The animals were randomly allocated to two feeding groups: protein restriction (15.9% CP, 0.73% lysine, 0.31% methionine) and control (19.4% CP, 0.9% lysine, 0.33% methionine). 12 animals / group and breed were slaughtered weekly during weeks 12-16, and meat and organ samples were collected. DPC were heavier than MLC ($p<0.001$). No differences were found between the feeding groups within the same breed regarding bodyweight and the relative gizzard, testicles and large intestine weight ($p>0.05$). Heavier relative liver ($p=0.011$) and small intestine ($p=0.009$) were detected in MLC fed the control diet. In DPC, the relative heart weight ($p<0.001$) was greater in the control group, while the caecum weight ($p<0.001$) was greater in the protein-restricted group. Both breeds had a greater dressing percentage in the protein-restricted groups (MLC $p<0.001$; DPC $p=0.031$). Both male DPC and MLC maintain bodyweight gain when fed balanced diets containing 15.9% crude protein from 8 to 16 weeks of age. However, protein restriction influenced relative organ weight.

Funding

We acknowledge funding by the Federal Ministry of Food and Agriculture (BMEL).

Effects of increasing standardized ileal digestible histidine and arginine to lysine ratios on growth performance of weanling pigs fed low or very low crude protein diets during the nursery period.

A.J. Galindo[1,2], D. Solà-Oriol[2], M. Verdú[3], M. Paniagua[1]
[1]Technical and R&D Department, Quimidroga SA, Barcelona, Spain. [2]Animal Nutrition and Welfare Service, Department of Animal and Food Science, Universitat Autònoma de Barcelona, Bellaterra, Spain. [3]Department of Animal Nutrition and Feed Industry, bonÀrea Agrupa, Guissona, Spain (mpaniagua@quimidroga.com)

Low crude protein (CP) diets supplemented with crystalline amino acids (AAs) are commonly used at weaning to reduce post-weaning diarrhea incidence and maintain intestinal health of piglets. These diets are typically balanced with essential AAs such as lysine (Lys), methionine, threonine, tryptophan or branched-chain AAs, to ensure animal requirements based on the ideal protein concept. However, some AAs like histidine (His) and arginine (Arg) are often overlooked despite their important functional roles in terms of physiological regulation (immune function, intestinal health and oxidative stress). Thus, we hypothesized that adding His and Arg above nutrient requirements in low CP diets would benefit weaned piglets. This study aimed to evaluate the impact of higher standardized ileal digestible (SID) His to Lys and SID Arg to Lys ratios in low and very low CP diets on the growth performance of weaned piglets. A total of 552 weaned piglets (DanBred × Pietrain; BW: 6.2 ± 0.67Kg) were allocated in two batches (n= 276). Within each batch, pigs were randomly assigned to 23 mixed-sex pens and four dietary treatments (5-6 replicates per treatment). During the pre-starter phase (PS: 0-15 days), diets included a very low CP diet (VLP:14% CP), VLP supplemented to achieve 38% SID His:Lys and 95% SID Arg:Lys ratios (VLPS), a low CP diet (LP: 16% CP), and LP supplemented until 38% SID His:Lys and 95% SID Arg:Lys ratios as well (LPS). In the starter phase (ST: 15-42 days), two diets of 18% CP were formulated with or without AAs according to PS supplemented treatments. BW and FI were measured on days 0, 15, 28, and 42 post-weaning and ADG, ADFI and F:G ratio were calculated for each period. Quantile analysis was also conducted to assess herd homogeneity by treatment. Data were analyzed using a 2×2 factorial arrangement with ANOVA, considering CP level and AAs supplementation as main factors, with batch included as a fixed effect. When significant effects were observed, post-hoc comparisons were performed using Tukey's test with R software. At the end of PS animals fed LPS had greater BW ($P<0.01$) than VLP treatment (8.40 vs. 7.95Kg). ADG was also higher ($P<0.01$) for LPS (147 vs. 117g/day) and F:G ratio was lower ($P<0.01$; 1.55 vs. 1.76) compared with VLP piglets. Quantile analysis showed significant BW differences ($P<0.05$) at the 75th percentile, with higher weight in VLPS (8.92Kg) vs. VLP (8.64Kg) and LPS (9.28Kg) vs. LP (8.84Kg). At the end of the trial, LPS 75th percentile was higher ($P<0.05$) than LP (22.40Kg Vs 21.5Kg). In conclusion, increasing SID histidine and arginine to lysine ratios above nutrient requirements in low CP diets improved growth performance of piglets during the pre-starter phase, particularly in the upper weight distribution.

Funding

This research was supported by the Catalan Industrial Doctorates Plan of the Department for Universities and Research, Ministry of Business and Knowledge of Generalitat de Catalunya (DI00094).

Characterizing whole body protein deposition and blood amino acid profiles of gestating sows in the first three weeks after breeding with or without recovery from previous lactation

A.F. Atoo[1], R.S. Samuel[1], L.A. Rodrigues[2], C.L. Levesque[1]
[1]South Dakota State University, Brookings, USA. [2]Zinpro Corporation, Eden Prairie, USA (crystal.levesque@sdstate.edu)

There is a paucity of data on the whole-body demand for amino acids (AA) in the period prior to pregnancy confirmation in gestating sows. To fully define AA requirements during gestation, whole-body AA demands during this period need to be characterized. This study aimed to characterize whole-body protein deposition and plasma AA profile from breeding to d21 of gestation in seven sows (203 ± 7.4 kg) in 2 consecutive parities. In parity 5, sows were bred approximately 53 days after weaning (the 'recovered' parity) and bred 4-5 days after weaning in the subsequent parity (the 'unrecovered' parity). Sows were allowed a 53-day recovery period after weaning and bred following estrus synchronization (the 'recovered' parity). Sows were fed 1.80 kg/d once daily of a gestation diet (3158 ME kcal/kg; 0.54 % SID Lys) to reflect common US industry sow feeding protocols. After farrowing, litters were standardized to 14 piglets, sows weaned 12.8 +/- 1.5 piglets. Nitrogen (N) balance collections (grab fecal and total urine via bladder catheters) were completed at 3 time points (d1-3, d11-13, d19-21) in early gestation of both the 'recovered' and 'unrecovered' parity. Blood was collected in the morning before feeding at least once in during each time point. Feed, urine, and feces were analyzed for N, and then N digestibility, N retention, and whole-body protein deposition (Pd) were calculated. Data was analyzed using PROC TTEST of SAS, comparing 'recovered' sows with 'unrecovered' sows. The N intake was 30 g/d in the 'recovered' and 31.8 g/d in the "unrecovered". The total tract digestibility of dry matter (95.2 vs 95.1%) and the urine output (kg/d) were not different ($P>0.05$) between the 'recovered' and the 'unrecovered' sows. Over the first 21 days of gestation, the 'recovered' sows had lower ($P<0.05$) N excreted in feces (2.48 vs 2.58 g/d) and urine (22.24 vs 38.92 g/d) excretion. The 'recovered' sows had positive N retention and Pd (5.21 and 32.53 g/d, respectively), while the 'unrecovered' sows had negative N retention and Pd (-9.59 and -59.93 g/d, respectively). Except for ILE, VAL, and PHE, the 'recovered' sows had greater ($P<0.05$) plasma concentration of essential AA in the first 21days of gestation. However, looking at the d1-3 time point, the 'unrecovered' sows had 82, 39, 35, 17, 50 percent higher ($P<0.01$) concentration of VAL, PHE, ILE, LEU, and TRP respectively. The difference in overall urinary N excreted, N retention and plasma AA in early gestation between 'recovered' and 'unrecovered' sows is likely reflective of mammary tissue involution. In conclusion, the contribution of mammary involution in the first 21 days of gestation may need to be considered to appropriately characterize the dietary AA needs of the recently bred female.

Funding

This study was funded by SDSU Agricultural Experiment Station Hatch Funds and USDA National Institute of Food and Agriculture (Hatch project: SD00H666-18) and Zinpro corporation.

The amino acid needs for an acute phase response in piglets: a theoretical approach

S. Millet[1], J. Teunen[2], N. Everaert[2]
[1]ILVO, Merelbeke-Melle, Belgium. [2]KU Leuven, Leuven, Belgium (nadia.everaert@kuleuven.be)

An infection or acute trauma triggers an acute phase response, characterized by elevated positive acute phase proteins (APP) and a decrease in negative APP. Reeds et al. (1994) suggested that nitrogen loss following such events results from increased demand for aromatic amino acids used in APP synthesis. Assuming muscle protein as the primary source, and considering the different amino acid compositions of muscle and APP, this can lead to excess release and oxidation of amino acids released during muscle degradation. Since publication, Reeds' paper has been cited to justify dietary amino acid adjustments during disease or trauma. However, it relied on human data and did not account for decreased negative APP. To explore this in piglet nutrition, we reviewed literature on APP levels in pigs under normal and disease conditions, and calculated amino acid amounts per kg of body weight in APP. Major positive APP in pigs under normal and acute conditions include pig-MAP (19.8 → 165 mg/kg BW), haptoglobin (13.2 → 148.5 mg/kg BW), serum amyloid A (0.61 → 49.5 mg/kg BW), C-reactive protein (0.165 → 2.64 mg/kg BW), and fibrinogen (92.4 → 132 mg/kg BW). The two values (x→y) represent concentrations under normal and acute conditions, respectively. Negative APP include albumin (759 → 528 mg/kg BW) and apolipoprotein-A1 (99 → 33 mg/kg BW). The amino acid ratios relative to lysine for APP under normal and acute conditions were calculated for Met + Cys (59 → 53), Thr (44 → 52), Trp (19 → 22), Ile (43 → 47), Leu (100 → 105), Val (52 → 63), His (39 → 39), Phe (66 → 63), Tyr (55 → 57), and Arg (66 → 71). Focusing solely on positive APP, the amino acid deposition in APP during an acute phase was calculated for a 20 kg piglet as 552 mg Lys, 266 mg Met + Cys, 400 mg Thr, 196 mg Trp, 318 mg Ile, 646 mg Leu, 503 mg Val, 244 mg His, 331 mg Phe, 372 mg Tyr, and 467 mg Arg. The latter values theoretically correspond with the SID amino acid content in 50-90g of a piglet diet containing 11g SID lys/kg, formulated based on an "ideal amino acid" composition (CVB, 2023). However, when taking into account amino acids released during breakdown of negative APP, the amino acid deposition in APP during an acute phase was calculated as -4.5 mg Lys, -116 mg Met + Cys, 158 mg Thr , 62 mg Trp, 75 mg Ile, 94 mg Leu, 212 mg Val, 0.1 mg His, - 61 mg Phe, 46 mg Tyr, and 93 mg Arg. In conclusion, while the amino acid profile necessary for APP deposition differs from that of muscle protein, the overall quantitative requirement appears to be comparatively modest relative to that for muscle accretion.

References

Reeds, P.J., Fjeld, C.R., Jahoor, F., 1994. The Journal of Nutrition 124, 906–910. https://doi.org/10.1093/jn/124.6.906
CVB, 2023. Booklet of Feeding Tables for Pigs. https://www.cvbdiervoeding.nl/bestand/10882/cvb-booklet-of-feeding-tables-for-pigs-2023def.pdf.ashx

Pullet energy intake from 16 to 25 weeks creates body weight differences leading to alterations in metabolic energy partitioning of broiler breeders during production

C. Umberson, D. Martinez, J.W. Wilkes, C.N. Coon
University of Arkansas, Fayetteville, AR, USA (ccoon@uark.edu)

A study was conducted to compare changes in feed allocation, dietary energy intake, source of dietary energy, and body weight gain curves (BWGC) from 16 to 25 weeks of age (WOA) on broiler breeder performance. A completely randomized design with a factorial arrangement 2 x 2 x 1 (2 studies x 2 growth curves x 1 negative control). Lipid turnover was measured via intraperitoneal infusion of deuterium oxide (99%) ^2H_2O achieving 2.5% atom percent excess (APE), 5% deuterium water was supplemented for drinking water to maintain 2.5% APE. Breeders 4/age/treatment (trt) were euthanized by CO_2 asphyxiation, fat pad was sampled 24 hours post-infusion for synthesis and 1 bird was sampled 7 days post infusion for triglyceride degradation. Protein turnover was measured by infusion of isotopic ^{15}N phenylalanine in the brachial vein, 4 breeders/age/trt were infused and euthanized by CO_2 asphyxiation. Synthesis was determined by ^{15}N phenylalanine incorporation in mixed skeletal protein, protein degradation was determined by loss of 3-methylhistidine. Body composition and egg quality were measured using dual energy x-ray absorptiometry; body crude protein, ash, and fat content/trt was determined by scanning 10 birds/trt/age. Low BWGC trts peaked in %egg production (EP) at 29 WOA but produced significantly less total and %hen-day egg numbers compared to high/medium BWGC trts over 20-wk production period ($p<0.05$). Low BWGC breeders did not achieve a minimum of 700 g of whole-body crude protein at 25 WOA (1st egg) compared to the high/medium BWGC trts. The Low BWGC pullets diverted energy utilization from EP to body composition building. High/Medium BWGC trts, regardless of energy intake and source of energy, peaked higher in protein fractional breakdown rates (FBR, 20-25%) to maintain EP in post-peak production compared to Low BWGC ($p<0.05$). High BWGC trts did not mobilize fat pad efficiently, relying upon protein FBR to support EP. Medium BWGC breeders relied on a combination of protein degradation and lipid degradation rates to maintain high EP and persistency of lay with an egg production index of 117 at 43 WOA. Energy intake affects body weight altering body composition, protein degradation rates, lipid degradation rates, egg production, and egg weights.

References

Vignale, K., Caldas, J.V., England, J.A., Boonsinchai, N., Sodsee, P., Putsakum, M., Pollock, E.D., Dridi, S., Coon, C.N., 2016. Poultry Science 0, 1–9. https://doi.org/10.3382/ps/pew369

Maharjan, P., Beitia, A., Weil, J., Suesuttajit, N., Hilton, K., Caldas, J.V., Umberson, C., Martinez, D., Kong, B., Owens, C.M., Coon, C., 2021. Poultry Science 100, 101092. https://doi.org/10.1016/j.psj.2021.101092

Funding

The research was funded by Cobb-Vantress and Tyson Foods.

Long-term influence of dietary lysine and energy on laying hen performance, body composition and metabolism from 0 until 110 weeks of age

L.M. van Eck[1,2], I.J. Carvalhido[1], H. Chen[1], H. Enting[1], R. Kwakkel[2]
[1]Cargill Animal Nutrition, Velddriel, Netherlands. [2]Wageningen University, Wageningen, Netherlands (lieske_van_eck@cargill.com)

The amount and ratio of protein and energy fed during rearing and lay influences the growth curve, body composition and laying persistency of laying hens (van Eck et al., 2024). We therefore aimed to study the influence of Apparent Fecal Digestible (AFD) Lys level in the rearing phase (high and low) and Metabolizable Energy (ME) Lay level and source in the laying phase (Reducing Energy over time with High Oil = REHO; Constant Energy High Oil = CE; Reducing Energy over time with Low Oil = RELO) on growth, body composition and laying persistency in a 2x3 factorial design. Per treatment, 240 one-day-old Dekalb White pullets were randomly divided over 8 pens. The experiment lasted until 110 weeks of age. BW was measured every 4 weeks, body composition by dissection of one hen per pen (breast weight, fat pad weight and liver weight) was measured every 8 weeks, feed intake and egg weight on a weekly basis and eggs were collected daily. Data were subjected to mixed model analyses. The egg production curve was modelled using a non-linear regression function. In general, there were no interaction effects ($P>0.05$) so main effects will be discussed. Feeding a low AFD Lys diet during rearing reduced feed intake, AFD Lys and ME intake ($P<0.01$) and resulted in long-term lower body weights (starting in week 5 until week 100; $P<0.05$ in most weeks). Egg mass production of pullets fed low AFD Lys was higher in week 44 to 72 ($P<0.05$) and 72 to 100 ($P=0.056$), while laying persistency was reduced, indicated by a longer peak production phase but a higher slope of decline after week 86 (modelled data; $P<0.05$). During lay, hens fed the CE diets had the lowest feed intake and AFD Lys intake from week 20 onwards ($P<0.05$), with a similar ME Lay intake, except for week 28 to 44, in which ME Lay intake was higher compared to the RELO diet ($P<0.05$). Egg weights of hens fed RELO diets were significantly lower from week 20 onwards ($P<0.05$). The peak production phase was on average 3 weeks longer for hens fed REHO and RELO diets compared to hens fed CE diets ($P<0.05$), while the slope of decline after week 85 was smaller for hens fed the REHO and CE diets ($P<0.05$). So reducing ME Lay and thereby increasing AFD Lys intake seems beneficial for maintaining a peak production phase, while providing a high oil diet increased egg weights and laying persistency. The latter could be related to impact on liver function and *de novo* lipoprotein production for yolk formation, indicated by heavier relative liver weights, with a higher crude fat content, of hens fed the RELO diets in most time periods ($P<0.05$).

Reference

van Eck, L., Chen, H., Carvalhido, I., Enting, H., Kwakkel, R., 2024. Poultry Science 103, 104124. https://doi.org/10.1016/j.psj.2024.104124

Response to dietary amino acid and energy is dependent on breed, species and age: comparative analysis of six similar central composite design experiments in broilers and turkeys

W. Lambert, T. Bruneau, F. Hervo, P. Moquet
Mixscience, Bruz, France (william.lambert@mixscience.eu)

Providing dietary metabolizable energy (ME) and amino acid (AA) as close as possible of the animal requirements is a prerequisite to maintain optimal performance, competitiveness and welfare of poultry birds. New trial methodologies have been lately applied in poultry nutrition such as central composite design (CCD) experiments to better quantify the complex responses to dietary nutrients and their interaction. Six recent CCD trials have been compiled to compare the dietary energy and amino acid responses of Ross 308 broilers (0-21d, 21-35d and 31-45d), Redbro slower growing broilers (14-31d and 31-48d) and Aviagen premium turkeys (81-122d). All trials have been performed in the same experimental station, with male birds and with the same experimental formulation strategies where ME increase was driven by fat content and AA increase was driven by soybean meal content. Interaction between dietary ME and dietary AA was tested for each performance criteria and for each trial. Interestingly, interaction was found for ADG but only for Ross 308 broilers from 0 to 21d and from 21 35d. In the two experiments, ADG was not affected by dietary ME when AA was low but ADG was increased by ME when AA was higher, leading to optimal growth in high ME and high AA conditions. Regarding ADFI, Ross 308 broilers did not regulate their intake on dietary ME and increased their ME intake with higher ME levels while Redbro broilers and turkeys reduced their ADFI when ME increased, leading to similar ME intake whatever the dietary ME (kcal/d). Increasing dietary AA either did not influence feed intake (Redbro) or increased ADFI (turkeys) and for Ross 308, the effect seemed to be dependent on age with increased ADFI in young broilers and decreased ADFI in older broilers. For ADG, increasing ME seemed to have no effect or a marginal effect on growth, except in Ross 308 broilers < 35d with increased ADG in high AA context. In all trials, ADG was increased with dietary AA. Regarding feed efficiency, FCR was decreased by both dietary ME and AA in an independent manner. Breast meat yields were consistently improved with dietary AA while the effect of dietary ME on breast meat yields was negative in Ross 308 broilers and turkeys. This methodology allows to model the performance response to dietary ME and AA for each poultry specie. Due to intensive genetic selection for rapid growth, modern fast-growing broiler strains such as Ross 308 exhibit a gowth pattern that is predominantly driven by AA intake. This selection pressure may have attenuated their capacity to regulate feed intake, particularly when compared to other genotypes such as Redbro or turkeys. Further research is warranted to elucidate the underlying mechanisms governing feed intake regulation in poultry birds.

Impact of glycine and serine supplementation on growth and nitrogen metabolism in broiler chickens with varying betaine supply

H. Philippi[1], W. Siegert[2], J. van Milgen[3], Y. Mercier[4], M. Rodehutscord[1]
[1]University of Hohenheim, Institute of Animal Science, Stuttgart, Germany. [2]University of Göttingen, Department of Animal Sciences, Göttingen, Germany. [3]INRAE, Institut Agro, Saint-Gilles, France. [4]Adisseo, European Labs for Innovation, Science & Expertise (ELISE), Lyon, France (hanna.philippi@uni-hohenheim.de)

Dietary glycine (Gly) is typically assessed alongside serine (Ser) at an equimolar level (Gly_{equi}) due to their reversible metabolic conversion, which involves 1-carbon units (1C-units). However, Ser differs from Gly in the molar quantity of 1C-units it can produce, and Gly can also take up 1C-units. This study aimed to investigate whether the availability of excess 1C-units influences the utilisation of dietary Gly and Ser. Maize-soybean meal-based diets (15% CP) were formulated to contain 105% of essential amino acid concentrations recommended by the Gesellschaft für Ernährungsphysiologie (GfE, 1999). Experimental diets were supplemented with 0 or 2 g/kg betaine (Bet-, Bet+) as source of 1C-units. Diets were formulated to contain 10.0 g Gly_{equi}/kg DM (no Gly or Ser supplementation (GlySer-)), 13.5 g Gly_{equi}/kg DM (from either Gly (Gly+) or Ser (Ser+) supplementation), or 17 g Gly_{equi}/kg DM (from either Gly (Gly++) or Ser (Ser++) supplementation), resulting in 10 experimental diets. 700 broilers were raised in floor pens and fed with a commercial starter. On d7, birds were allocated to 70 metabolism units, with 10 birds per unit. Each diet was tested in 7 replicates and fed from d7–21. Total excreta were collected twice daily on d18–21 to determine N-accretion and nitrogen utilization efficiency (NUE). Treatment effects were statistically analysed by one-way ANOVA. In Bet- treatments, Gly+ resulted in a greater increase in average daily gain (ADG d7-21) and average daily feed intake (ADFI d7-21) compared to Ser+ (P=0.017). In Bet+ treatments, ADG and ADFI remained similar, regardless of whether Gly or Ser was supplemented (P>0.050). Bet-Gly+ and Bet-Ser+ decreased feed conversion ratio (FCR) compared to Bet-GlySer- (P<0.001). In Bet+ treatments, where FCR was lower, only Gly++ and Ser++ reduced FCR compared to Bet+GlySer- (P<0.009). Gly supplementation increased N-accretion (d 18-21) in Bet- treatments compared to GlySer- and compared to Ser+(+) (P<0.001). In Bet+ treatments, neither Gly nor Ser supplementation led to an increase in N-accretion compared to Bet+GlySer- (P>0.050). In Bet- treatments, Ser++ had a lower NUE compared to GlySer- (P=0.046). Both levels of Gly and Ser+ maintained NUE at 77%, which was similar to the GlySer- treatment (P>0.050). In Bet+ treatments, neither Gly nor Ser supplementation affected NUE compared to the Bet+GlySer- treatment (P>0.050). NUE was lower for Bet+Gly+(+) and Bet+Ser+ compared to their Bet- counterparts (P≤0.048). In low crude protein Bet- diets, Gly supplementation appears necessary to counteract reduced growth and N-accretion and cannot be replaced by Ser supplementation. However, betaine supplementation appears to equalise the utilisation of Gly and Ser. Whether betaine supplementation contributed to the equimolar utilisation of Gly and Ser directly through its role as 1C-unit donor or via another mechanisms requires further elucidation.

Reference

GfE (Gesellschaft für Ernährungsphysiologie), 1999. Empfehlungen zur Energie- und Nährstoffversorgung der Legehennen und Masthühner (Broiler). DLG-Verlag, Frankfurt am Main, Germany

Required dietary digestible lysine content for maximum growth performance in weaned piglets

N.E. Manzke[1], P. Pluk[1], A.J.M. Jansman[2], P. Bikker[2]
[1]Cargill Animal Nutrition, Velddriel, Netherlands. [2]Wageningen University & Research, Wageningen, Netherlands (Naiana_Manzke@cargill.com)

In a review of 41 dose-response studies, Goethals et al. (2025) found that increasing dietary lysine (LYS) content led to a linear increase in average daily gain (ADG) and feed efficiency (G:F). Regression analysis suggested that standardized ileal digestible (SID) LYS requirement for weaned pigs may exceed 1.3 g SID LYS/MJ net energy (NE), but did not provide a more precise estimate of the LYS requirements to optimize performance. Therefore, this study aimed to determine the optimal SID LYS content for growth performance in weaned pigs. A total of 288 pigs (TN Select x TN 70), weaned at 23 days old, were assigned to six dietary treatments 10 days post-weaning. Each treatment had eight pens, with six pigs per pen (mean initial body weight (BW) 10.3 kg). The calculated dietary SID LYS content ranged from 7.0 to 17.0 g/kg in six equidistant steps at a NE level of 10 MJ/kg. All other essential amino acids were included in a ratio of 105% of CVB-recommendations relative to LYS content and, therefore, increased with higher dietary LYS levels. Calculated crude protein (CP) content increased from 114 to 240 g/kg. Diets were fed from 10 to 39 days post-weaning, with feed intake and growth performance recorded weekly. Ileal digestibility of CP and amino acids was assessed using a parallel nitrogen balance study with ileal-cannulated pigs, which included pigs fed low, medium, and high SID LYS content diets. Statistical analysis used the lme4 package in R, with pen as the experimental unit, dietary SID LYS as a fixed factor, and weight blocks as a random factor. A broken-line regression model (minpack.LM package in R) estimated SID LYS content at the breakpoints using one segment with a variable slope and the other as a plateau. Feed intake increased linearly or quadratically ($P<0.05$) with increasing dietary LYS content in weekly periods. Average daily gain and G:F increased both linearly ($P<0.0001$) and quadratically ($P<0.01$) across all periods. To maximize ADG, the estimated dietary SID LYS requirement decreased from 13.3 to 10.1 g/kg from the first to the fourth week, averaging 11.3 g/kg over the total period. To maximize G:F, the SID LYS requirement declined from 13.8 to 12.2 g/kg, with an average of 12.5 g/kg over the four experimental weeks. Final BW increased from 24.1 to 31.6 kg with the increase in dietary SID LYS, averaging 11.5 g/kg of SID LYS over the four-week period to reach the maximum BW. Higher dietary LYS and CP reduced fecal consistency in the first two weeks, though diarrhea was not observed. These findings help to optimize SID LYS levels for maximum growth performance of weaned pigs.

Reference

Goethals, S., Bikker, P., Rijpert, J.H.M., Ampe, B., Spek, J.W., Millet, S., 2025. Animal 19, 101323. https://doi.org/10.1016/j.animal.2024.101323

Funding

This research was conducted within the public private partnership “Feed4Foodure-III; Sustainable animal nutrition in circular agri-food systems” (TKI-Agrifood LWV20203), partially funded by the Ministry of Agriculture, Fisheries, Food Security and Nature (project BO-55-001-015), and partly by “Vereniging Diervoederonderzoek Nederland”.

Formulating diets with productive energy (Arkansas Net Energy) improves carcass yield and economics in broilers compared with apparent metabolizable energy

D. Martinez, C. Umberson, E. Sheikhsamani, S. Johnson-Young, C. Owens, C.N. Coon
University of Arkansas, Fayetteville, Arkansas, USA (ccoon@uark.edu)

Productive energy (PE) is net energy (NE) for gain (NEg) + NE for maintenance (NEm). NEg and NEm are measured with Dual-Energy X-Ray Absorptiometry and indirect calorimetry, respectively. PE predicts BW gain (BWG), feed intake (FI), feed efficiency (FEF; BWG/FI), body composition, processing weights, and economics better than N-corrected apparent metabolizable energy (AMEn) in broilers. The experiment assessed the impact of formulating broiler diets with PE. 18 floor pens were assigned to each of five feeding phases and were independent experiments (each phase started with fresh birds). Pens in phases 1 to 5 hold reducing number of birds (30 to 16 birds each). Treatments consisted of diets formulated with AMEn (T1) or with PE at 83% (T2) or 92% (T3) of PE requirement to obtain a linear response. IN each phase, BW, BWG, FI, FEF, and mortality (MO) were determined. Gompertz curve first derivative was used fit BWG data, and cumulative BW (cBW) and BWG (cBWG) were obtained. Cumulative FI (cFI) was obtained based on FEF and cBWG and cumulative European Broiler Index (EBI) were obtained. At 56 d, 148 birds from 9 additional pens, receiving treatment diets continuously (1-56 d), were processed. Carcass (CAR), leg quarters (LQU), wings (WIN), and breast meat + wing (BPW) yields (%) and woody breast (WB) and white stripping (WS) indexes (1-3 scores) were measured. Feeding cost per bird (FCB), per kg BW (FCW), and per kg carcass (FCC) were calculated. Carcass market value (CMV, \$/bird), gross profit (GPR; \$/bird; CMV – FCB), and feed return of investment (ROI; GPR/FCB) were determined. Data was adjusted to processing weights and mean response at 100% PE requirement (T4) was obtained with the regression of treatments T2 and T3. T4 data was obtained with bootstrapping and Monte Carlo procedures in JMP Pro 16. ANOVA and Student's t-test were used. Feeding PE diets continuously produced higher BW ($P=0.01$) than the BW obtained with the independent phases, which agrees with an improved protein synthesis rate with higher diet PE. Linear positive effects were observed on cFCR, EBI, CAR, LQU, WIN, FCC, and ROI ($P\leq0.05$) with increasing PE densities (T2 to T3). Higher cBW, cBWG, cFEF, EBI, CAR, LQU, FCC, CMV ($P<0.001$), GPR ($P=0.002$), and ROI values were observed in T4 compared with T1 ($P < 0.001$). No effect of increasing PE was observed on WB and WS ($P>0.05$). PE formulated diets at 100% had higher soybean meal inclusion rates. PE at 83%, but not at 92%, showed lower FEF than AMEn-fed birds. In conclusion, PE shows potential to improve production efficiency and economics of broiler production compared to AMEn. PE increases carcass yield, and reduces formula and feeding costs with no negative impact on meat quality.

References

Martinez, D.A., Weil, J.T., Suesuttajit, N., Umberson, C., Scott, A., Coon, C.N., 2022. Animals (Basel) 12, 2706. https://doi.org/10.3390/ani12192706
Maharjan, P., Martinez, D.A., Weil, J., Hilton, K.M., Mullenix, G., Beitia, A., Suesuttajit, N., Umberson, C., Coon, C.N., 2021. Animal 15(Supplement 1), 100284. https://doi.org/10.1016/j.animal.2021.100284

Funding

The research was funded by the United Soybean Board

Sows losing backfat thickness during lactation improve productivity and modify the energy balance, compared with no losing sows

M. Fornós, E. López, J. Gasa, D. Solà-Oriol
Animal Nutrition and Welfare Service (SNiBA), Departament de Ciencia Animal y de los Alimentos, Universidad Autónoma de Barcelona, Bellaterra 08193, Spain (marta.fornos@uab.cat)

During lactation, most sows mobilize their own body tissue, energy, and nutrients, especially during the first week or ten days post-farrowing. Backfat thickness (BF) is partially related to the sow's body energy content, although it does not appear to be an accurate predictor of the sow's energy balance. The aim of the present study was to explore the influence of BF loss on lactation productivity and energy balance of sows. The present experiment was performed in a commercial farm where a total of 83 (Landrace x Yorkshire) multiparous sows (cycles 3 to 6) were monitored during lactation. Sows' live body weight (BW) and BF were recorded upon entering the farrowing crates and at weaning. Sows' BW at farrowing was estimated based on BW upon entering the barn, days to farrowing, and litter size. Sows' feed consumption was recorded daily, and individual piglets' weights were recorded at birth and at 2, 7 and 22 days of age. An average of 16 piglets were allocated to each sow at cross fostering (day 0), and the weaned piglets were recorded after 22 days of lactation. From the collected data, litter weight and growth were calculated, and energy, fat, and protein balance in the body of sows were estimated, using the BW and BF difference between farrowing and weaning. To analyze the results, the sows were distributed into two groups: sows losing BF (BFL, n = 42) and sows not losing BF (BFNL, n = 41) during the whole lactation. Data were analyzed with ANOVA using PROC GLIMMIX, a statistical package of SAS, and least squares means (LSMeans) with Tukey adjustment were used to compare treatment levels. BF at farrowing was considered as a co-variate. BFL sows weaned more piglets (14.3 vs 12.7; $p = 0.0012$) and registered a lower piglet mortality (10.0 vs 20.3%; $p < 0.0007$) than BFNL sows. BFL sows also weaned heavier litters (76.9 vs 60.1 Kg; $p < 0.0001$), performed better litter gain (54.7 vs 41.8 Kg; $p < 0.0001$), and weaned heavier piglets (5.4 vs 4.7 kg/piglet; $p = 0.0002$). By contrast, BFNL sows registered a numerically 6.5% higher feed intake (7.7 vs 8.2 kg/d; $p = 0.169$), resulting in a higher BW gain (0.09 vs 23.42 kg; $p < 0.0001$) and an increased body energy balance (-2.8 vs 104.4 Mcal; $p < 0.0001$) compared to BFL sows. BFNL sows also had a higher body protein (1.6 vs 4.7 kg, $p < 0.0001$) and body fat balance (-1.3 vs 7.7 Kg; $p < 0.0001$). In conclusion, sows that lose BF during lactation produce heavier litters and piglets and are more efficient compared to sows that do not lose BF. These results suggest that fat mobilization is the key issue influencing lactation performance efficiency.

Adult male cats consuming diets ranging from 110-205% of the recommended allowance for crude protein had altered digestible energy, but not metabolizable and net energy

S. F. Anan[1], E. Labussière[2], D.J. Seymour[1,3], A.K. Shoveller[1]
[1]University of Guelph, Guelph, Canada. [2]Pegase, INRAE, Saint-Gilles, France. [3]Trouw Nutrition, Boxmeer, Netherlands (sanan@uoguelph.ca)

Digestible (DE) (Kendal et al., 1983) and metabolizable energy (ME) (Hashimoto et al., 1995) have previously been quantified in total collection studies conducted in cats; however, the losses occurring through heat production and the resulting net energy (NE) have not been estimated. As such, we aimed to measure NE in diets by measuring DE, ME and total postprandial heat increment of feeding (HIF) using fecal, urine and gas exchange measurements, respectively. Two extreme experimental diets containing 110% (22% CP DM) and 205% (41% CP DM) of the recommended allowance were formulated and subsequently mixed to form four more intermediate treatments (CP = 25%; 26%; 29%; 36% DM). The formulated diets also included titanium oxide (TiO_2) as an indigestible marker. Treatments were fed in a replicated 6x6 Latin square design to twelve cats over a 14-day period. On d13 cats were placed in calorimetry chambers to measure feed intake, VO_2, VCO_2 and collect urine and feces over 24-hours. Feed, fecal and urinary energy were measured using bomb calorimetry for estimating energy partitioning in gross energy (GE), DE, ME and NE. HIF was calculated as area under the curve of total energy expenditure using linear trapezoidal rule and resting fed metabolic rate (RFMR). Fecal Ti was used to evaluate apparent total tract energy digestibility. Percentage energy utilization was estimated from partitioned values. Data were analyzed in SAS using PROC GLIMMIX. Treatment had a significant effect on GE and DE on a body weight (BW) and feed intake basis. Cats consuming 36% CP had the greatest GE (63.7kcal/kg BW; $P = 0.01$) and DE (44.4kcal/kg BW; $P = 0.01$) but when corrected for feed intake, DE (392.0kcal/100g DM; $P = 0.02$) remained highest on 36% CP diet but GE was highest for 41% CP diet (558.8kcal/100g DM; $P < 0.01$). Cats fed 25% CP had lowest GE (59.7kcal/kg BW), but 22% CP had lowest GE on a feed intake basis (531.1kcal/100g DM). DE was lowest for both 22% (30.6kcal/kg BW) and 25% CP treatment (31.9kcal/kg BW; $P = 0.01$). ME (33.6kcal/kg BW; $P = 0.07$) and NE (27.5kcal/kg BW; $P = 0.09$) tended to be highest for 36% CP; however, ME (215.9-296.6kcal/100g DM; $P = 0.12$) and NE (167.8-241.1kcal/100g DM; $P = 0.14$) did not differ among diets on feed intake basis. DE as a percentage of GE tended to be highest in 36% CP (70.5%) and lowest for 22% CP (52.3%; $P = 0.07$) but there were no significant differences in other utilization values. This research provides the first reported values of NE in diets from low to high protein content for cats and should be considered to establish more accurate predictive algorithms for energy density. Overall, energetic efficiency remained similar across increasing crude protein treatments in cats.

References

Kendall, P.T., Blaza, S.E., Smith, P.M., 1983. Journal of Nutrition 113, 1946-1955. https://doi.org/10.1093/jn/113.10.1946

Hashimoto, M., Funaba, M., Ohshima, S., Abe, M., 1995. Experimental Animals 44, 23-28. https://doi.org/10.1538/expanim.44.23

Funding

The project was funded by Natural Sciences and Engineering Research Council of Canada (NSERC) Alliance grant and Petcurean Pet Nutrition. Additionally, experimental diets used in this research were formulated and manufactured by Petcurean.

Supplemental creatine lowered plasma BCAAs and tended to lower respiratory quotient, while precursors, Arg and Met did not lead to increased creatine in the cat

T. Richards[1], S. Banton[1], J.G. Pezzali[2], J. Saunders-Blades[3], A.K. Shoveller[1]
[1]University of Guelph, Guelph, Canada. [2]Kansas State University, Manhattan, USA. [3]Champion Pet Foods, Edmonton, Canada (ashovell@uoguelph.ca)

Creatine (Cr) is a naturally occurring nutrient found in animal-derived ingredients commonly used in pet food. It plays a key role in supporting energy metabolism, muscle strength and recovery, and brain health—benefits that are increasingly relevant as more pet foods start to focus on promoting healthy aging (Forbes et al., 2021). Since the body continuously uses and converts Cr to creatinine (CrN), regular dietary intake of Cr is essential to sustain these benefits. When dietary Cr intake is insufficient, it must be produced by the body, which requires the use of amino acids (AA), methionine (Met) and arginine (Arg) (Brosnan and Brosnan, 2016). These AA play an important role in supporting various biological functions, including protein synthesis and immune system regulation. Moreover, Met is often the first limiting AA in commercial cat diets, and cats have a greater requirement for Arg than omnivorous monogastric species. By reducing reliance on internal Cr synthesis, improved dietary strategies could (1) conserve essential AAs like Arg and Met for other physiological functions and/or (2) provide the aforementioned health benefits associated with Cr supplementation. Taken together, the objective of this work was to investigate the effects of supplemental Cr on plasma AA concentrations, urine Cr and CrN concentrations, and energy expenditure in cats. Using a balanced replicated incomplete Latin square design, cats (n=14) were fed three diets in random order: (1) a base kibble (control), and the base diet supplemented with (2) Cr and (3) Arg + Met. Each diet was fed for 14 days, blood was collected on d14 and AA were measured using UPLC, urine was collected on d12 and Cr and CrN were measured using UPLC coupled with QToF-MS, and 24h indirect calorimetry was performed on d14 to measure energy expenditure and respiratory quotient. Plasma Arg was greatest in cats fed the Arg+Met diet, followed by the Cr diet and then the control diet (*P<0.0001*), with no differences among treatments observed for plasma Met (*P=0.1816*). Plasma branched-chain AA (BCAA), leucine, isoleucine, and valine were greater in cats fed the control and Arg+Met diets compared to the Cr diet (*P=0.0009; P=0.0044; P=0.0173*, respectively). This may reflect an increased utilization of these BCAAs for muscle protein synthesis due to Cr supplementation, potentially supporting muscle repair and growth. Urine Cr and CrN were greater in cats fed the Cr diet compared to the control and Arg+Met diets (*P=0.0006; P<0.0001,* respectively), indicating that excess dietary Arg and Met is not used for Cr synthesis. The average respiratory quotient tended to be lower in cats fed the Cr diet compared to the control (*P=0.0991*), suggesting a shift towards fatty acid oxidation. These findings indicate that dietary Cr may support muscle growth and repair and body composition in cats.

References

Forbes, S.C., Candow, D.G., Ferreira, L.H.B., Souza-Junior, T.P., 2021. Journal of Dietary Supplements 19, 318-335. https://doi.org/10.1080/19390211.2021.1877232
Brosnan, M.E., Brosnan, J.T., 2016. Amino Acids 48, 1785-1791. https://doi.org/10.1007/s00726-016-2188-1

Funding

This research was funded by Champion Petfoods in partnership with NSERC Alliance and Mitacs Accelerate Programs.

Effects of dietary energy source and stress on equine gut permeability and inflammation

A.R. Bauman[1], J.B. May[2], E.N. Adam[3], M.J. Talley[1], S.N. Carr[1], E.S. Vanzant[1], V.J. Healy[1], K.R. McLeod[1]
[1]Department of Animal and Food Sciences, University of Kentucky, Lexington, USA. [2]Department of Plant and Soil Sciences, University of Kentucky, Lexington, USA. [3]Department of Veterinary Science, Maxwell H. Gluck Equine Research Center, University of Kentucky, Lexington, USA (kyle.mcleod@uky.edu)

Dietary energy sources and stress can disrupt gut homeostasis and affect whole-body energy and protein metabolism. Accordingly, we investigated the impact of diet and stress on gut permeability using 32 horses (2 blocks, n=16/block) randomly assigned to one of four isocaloric diets: 1) cracked corn (CC; 4 g starch·kg $BW^{-1}\cdot d^{-1}$ and 0.2 g fat·kg $BW^{-1}\cdot d^{-1}$), 2) puffed corn (PC; 4 g starch·kg $BW^{-1}\cdot d^{-1}$ and 0.2 g fat·kg $BW^{-1}\cdot d^{-1}$), 3) PC and soybean oil (MF; 2 g starch·kg $BW^{-1}\cdot d^{-1}$ and 0.6 g fat·kg $BW^{-1}\cdot d^{-1}$), or 4) PC and soybean oil (HF; 1 g starch·kg $BW^{-1}\cdot d^{-1}$ and 1.2 g fat·kg $BW^{-1}\cdot d^{-1}$). Horses were housed in outdoor dry lots and individually fed twice daily (free access to hay between meals) and exercised three times weekly. Experimental periods consisted of a baseline (hay only), diet adaptation (stepwise increase over 2 weeks), and a 7-week full-feeding treatment sub-period. The treatment sub-period was divided into diet alone (week 5) and diet plus stress (12 h; 20-min transit to individual stall housing during week 7). Gut permeability was measured using jugular blood appearance of non-metabolizable sugar analogues over 12 h following gastric dosing. The aqueous mixture contained fermentable (mannitol and lactulose, 40 and 70 mg·kg BW^{-1}, respectively) and non-fermentable (sucralose, 200 mg·kg BW^{-1}) analogues to distinguish small and large intestinal permeability. Post-prandial plasma glucose and insulin concentrations were determined at week 3 of the treatment sub-period over 420 min. Salivary cortisol concentrations and whole-blood gene expression of cytokines were determined before and during the stress challenge. Post-prandial plasma glucose and insulin concentrations increased ($P \leq 0.05$) above baseline at 30 minutes for all treatments. Glucose concentration returned ($P \geq 0.09$) to baseline for all treatments by 240 minutes, except PC which returned ($P=0.11$) at 360 minutes. Insulin concentration returned ($P \geq 0.08$) to baseline at 180, 300, and 420 minutes for HF, CC, and MF, respectively, while insulin did not return ($P \leq 0.05$) to baseline for PC. Area under the curve (AUC) for plasma sucralose was greater or tended to be greater for horses fed HF ($P=0.004$) and CC ($P=0.06$) compared with those fed PC; HF tended to be greater ($P=0.06$) than MF. Stress increased cortisol concentration ($P<0.001$), Il-1β and Il-6 gene expression ($P \leq 0.05$), and peak plasma concentration and AUC ($P \leq 0.02$) for all sugar analogues. These data indicate that gut permeability in horses is sensitive to hindgut starch supply and dietary fat, and that small intestinal permeability and systemic inflammation is highly responsive to psychological stress. Taken together, these findings suggest that diet and stress can influence gut permeability, which may translate to alterations in whole-body energy and protein metabolism.

Effects of cashew nutshell liquid on methane emission and nutritional use in sheep fed a high-concentrate diet

S. Hisadomi, T. Obitsu, Y. Kuroki, H. Ogura, R. Yamagiwa, T. Sugino, Y. Inabu
Graduate School of Integrated Sciences for Life, Hiroshima University, Higashi-Hiroshima, Japan (d241758@hiroshima-u.ac.jp)

Dietary supplementation of cashew nutshell liquid (CNSL) containing anacardic acid to ruminants reduces methane emissions and enhances ruminal propionate production through the transition of the ruminal microbiota composition (Shinkai *et al.*, 2012). The changes in ruminal fermentation induced by CNSL supplementation may affect nutrient metabolism in ruminants depending on the types of basal diets, which also affect microbial fermentation in the rumen. Therefore, this study aimed to clarify the effects of CNSL on methane emission and nutrient use in sheep fed a high-concentrate diet. The experiment was arranged as a crossover design comprising two experimental periods, with 21 days in each period. Six mature Suffolk wethers (BW, 59.1±1.3 kg) were divided in two groups, with three animals in each group. One group was fed a basal diet with a forage: concentrate ratio of 30:70 (75% TDN and 12% crude protein on a dry matter (DM) basis) without supplementation in the first period, then, the wethers were fed the basal diet with 0.37% of CNSL as a DM basis in the following period. For the other group, the feeding treatments were administered in the reverse order. Sheep were fed the diets at 1.5 times maintenance energy level, in two equal portions at 08:30 and 17:30. In the third week of each period, the ruminal fermentation profile, *in vitro* methane production, nutrient digestibility, nitrogen balance, and plasma metabolite profile were measured. DM intake, apparent DM digestibility and total VFA concentration in the rumen were not different between sheep fed with or without CNSL supplementation, hence CNSL did not affect the nutrient intakes of sheep. Supplementation of CNSL decreased ($P<0.01$) *in vitro* methane production and the proportion of acetate in the rumen, while it increased ($P<0.01$) ruminal propionate proportion as we expected. Additionally, CNSL supplementation increased ($P<0.01$) plasma glucose concentration but reduced ($P<0.01$) total ketone-body concentration. Furthermore, CNSL supplementation reduced ($P<0.01$) the plasma concentration of triglyceride and cholesterol. Meanwhile, CNSL supplementation did not affect ruminal ammonia nitrogen concentration, and the excretion of faecal and urinary nitrogen and urinary allantoin. In conclusion, dietary CNSL supplementation, which reduces methane production and enhances propionate production in the rumen, shows potential to affect energy metabolism by altering gluconeogenesis, ketogenesis, and lipid metabolism in sheep fed high-concentrate diets.

Reference

Shinkai, T., Enishi, O., Mitsumori, M., Higuchi, K., Kobayashi, Y., Takenaka, A., Nagashima, K., Mochizuki, M., Kobayashi, Y., 2012. Journal of Dairy Science 95, 5308–5316. https://doi.org/10.3168/jds.2012-5554

Funding

This work was supported by JST SPRING, Grant Number JPMJSP2132.

Effect of forage source and 3-nitrooxypropanol on milk production, rumen fermentation and methane production of dairy cows

N. Rummukainen, S. Salin, P. Rissanen, A. Halmemies-Beauchet-Filleau, M. Lamminen, A. Vanhatalo, T. Kokkonen
University of Helsinki, Helsinki, Finland (netta.rummukainen@helsinki.fi)

Previous studies have suggested that neutral detergent fiber (NDF) and forage source may affect the efficiency of feed additive 3-nitrooxypropanol (3-NOP) in dairy cow diets. We studied the effect of 3-NOP on ruminal methane production, milk yield (MY) and feed intake in dairy cows using two different forages, whole crop barley (BS) and grass silage (GS) differing in fiber and starch composition. This experiment was conducted using a switch-back design with three periods, each lasting four weeks. During the first and third period, cows received a mixture of BS and GS in a 50:50 ratio [on dry matter (DM) basis]. In the second period, cows received GS as the only forage. Half of the cows were fed control concentrate and another half concentrate containing 3-NOP additive (Bovaer, DSM Nutritional Products Ltd). In total, 20 Finnish Ayrshire cows were assigned to pairs based on parity and previous milk and methane production. Cows in each pair were randomly assigned to one of the two concentrate treatments, control or 3-NOP treatment. Cows received the same concentrate mixture throughout all periods. Concentrate mixtures and forages were fed ad libitum as a partial mixed ration (PMR), containing 70% forage and 30% concentrate (on DM basis). In addition, cows received a fixed amount of pelletized robot feed from milking robot (4 kg/d for primiparous and 4.5 kg/d for multiparous cows). Feed intake, MY and gas exchange (GreenFeed, C-Lock Inc) were recorded, and milk composition, spot fecal samples and rumen fluid samples were collected during the last 10 days of each period. Diet that contained BS had higher starch content (19.4% vs. 12.7% of DM) and lower crude protein content (14.8% vs. 16.8%) than GS. The indigestible NDF (iNDF) content in BS was three times higher than that in GS, and as a result, dietary iNDF content was higher in the first and third period. Dietary 3-NOP concentration was 64 mg/kg DM. Feed intake (kg/d) was reduced by 6.7% and energy corrected milk yield (kg/d) by 4% when cows received 3-NOP, and the reduction was greater when the diet contained BS. Adding 3-NOP to the diet tended to increase the molar proportion of propionate in the rumen and reduced that of acetate. Methane production (g/d) was reduced in each period by 3-NOP, but the effect was greater with BS (25.5%) compared to GS (19%). Using 3-NOP increased hydrogen emissions. Although 3-NOP reduced methane emissions with both forages, at least part of the reduction is explained by the decreased intake. The cause for reduced intake is unclear, though it may be partly explained by the increased partial pressure of hydrogen and molar proportion of propionate in the rumen, acting as satiety signals.

Funding

This study was a part of a IRMA–project: Climate smart feeding solutions for Finnish milk production sector (Ministry of Agriculture and Forestry of Finland, Helsinki, Finland; grant number VN/20638/2022-MMM-4)

Effect of beef cattle breed and stocking rate on enteric methane emission intensity and rumen volatile fatty acid profiles of beef heifers grazing a naturalized pasture in Western Canada

A. Macias Franco[1], A.E. Moreira da Silva[1], W. Hao[1], V. Ramirez Sepulveda[1], M. Londono-Mendez[1], S. Lasso Ramirez[1], C. Fitzsimmons[1,2], E. Santos[1], K. Wood[3], G.O. Ribeiro[4], S. Terry[5], G. Medeiros da Silva[1]
[1]University of Alberta, Edmonton, Canada. [2]Agriculture and Agrifood Canada, Lacombe, Canada. [3]University of Guelph, Guelph, Canada. [4]University of Saskatchewan, Saskatoon, Canada. [5]Lethbridge Research and Development Center; Agriculture and Agrifood Canada, Lethbridge, Canada (gleise@ualberta.ca)

Increasing beef production to meet a growing protein demand while mitigating enteric methane emissions is crucial to ensure the long-term sustainability of this industry. This study evaluated the effects of two stocking rates on enteric emissions and rumen volatile fatty acid (VFA) profiles of beef heifers grazing a naturalized pasture in Western Canada. A total of 42 heifers (60% Angus or 40% Hays Converter) were allocated to either a high (2.3 animal unit months [AUM]/hectare, target-70% biomass utilization; n = 28) or low (1.1 AUM/hectare, target-30% biomass utilization; n = 14) stocking rate, with each breed represented in both treatments. The animals grazed naturalized pastures dominated by *Poa pratensis* L. (46%), *Bromus inermis Leyss.* (29%), and *Symphoricarpos albus* (L.) Blake (9%) divided in four 17-ha paddocks for 100 days, with only 2 pastures grazed at a time. Enteric methane (CH_4) and carbon dioxide (CO_2) emissions were continuously monitored using one C-Lock Inc. GreenFeed head-chamber unit (per paddock), average daily gain (ADG), and rumen fluid was sampled through esophageal tubing for VFA analysis on days 0, 60, and 100. Forage biomass and chemical composition were assessed biweekly. Linear mixed models with repeated measures were used to evaluate the effects of stocking rate, breed and their interaction, and time, with paddock nested within time as a random factor. Heifer CH_4 emissions (g day^{-1}) were not statistically different by stocking rate ($P = 0.403$), but were higher for Hays Converter than Angus (186 vs 178; $P = 0.02$). ADG-adjusted CH_4 decreased throughout the experiment ($P < 0.001$), was lower for Hays Converter animals (189 vs 208; $P < 0.001$), and tended to be lower at the lower stocking rate (188 vs 209; $P = 0.057$). CO_2 emissions (g day^{-1}) were not significantly affected by treatment or breed; however, ADG-adjusted CO_2 decreased over time ($P < 0.001$) and was lower for Hays Converter than Angus (7345 vs 8065; $P < 0.001$). A stocking rate by breed interaction was detected for feeder visits ($P < 0.001$) with more visits for high stocking rates, but Hays Converter increasing visits at low stocking rates. Total VFA, acetic, and propionic acid increased over time ($P < 0.001$) and were greater for Angus ($P < 0.001$) and at low stocking rates ($P = 0.041$). Acetate-to-propionate and individual VFA (acetic, propionic, isobutyric, valeric, and isovaleric) showed an interaction between stocking rate and breed ($P < 0.05$), where Angus and low stocking rates were higher. Preliminary results suggest that low stocking rates may differentially affect enteric emissions and fermentation profiles, and that breed-specific differences exist between Hays Converter and Angus heifers in extensive pasture systems. This study will continue over two more years to confirm these findings.

Funding
This project was financed under the Beef Cattle Research Council (ENV.11.21C), and the primary authors' funding through the Izaak Walton Killam Postdoctoral Fellowship of the University of Alberta, Edmonton.

Dietary protein source modification in pigs: effects on ammonia emissions and methane production

I. Kaikat[1], L. Piquer[2], A. Cerisuelo[2], D. Solà-Oriol[1], J. Soder-Walz[3], P. Blánquez[3], J.F. Pérez[1]
[1]Animal Nutrition and Welfare Service (SNiBA), Department of Animal and Food Science, Universitat Autònoma de Barcelona (UAB), Bellaterra, Spain. [2]Centro de Investigación y Tecnología Animal (CITA), Instituto Valenciano de Investigaciones Agrarias (IVIA), Segorbe, Spain. [3]Chemical, Biological and Environmental Engineering Department, Escola d'Enginyeria, Universitat Autònoma de Barcelona (UAB), Bellaterra, Spain (ibtissam.kaikat@uab.cat)

Pig production is a significant contributor to environmental emissions, with ammonia (NH_3) and methane (CH_4) being major pollutants from slurry. Dietary strategies targeting nitrogen (N) partitioning can help mitigate these emissions. This study evaluated the impact of replacing soybean meal (SBM) with rapeseed meal (RSM) and sunflower meal (SFM) on N balance, slurry composition, and gaseous emissions in pigs. Three experimental diets were formulated to contain 16% of SBM, RSM, or SFM and fed to 12 growing pigs ((PxD) x (LDxLW); 27.0 ± 1.17 kg BW) over four 14-day periods following a Latin square design. Each pig received all three diets over the course of the study. After a 7-day adaptation, total feces and urine were collected per pig for three days and proportionally mixed to generate final slurry for monitoring NH_3 emission using a dynamic chamber system with acid traps, following Beccaccia et al. (2015), and biochemical CH_4 potential (BMP) using batch incubation at 35 °C for 25 days with anaerobic inoculum, following Martín-González et al. (2010). Additional urine and feces samples were collected for two days to determine N balance. Slurry excretion per animal (kg/day) and per kg of diet (kg slurry/kg diet) were similar across diets. Total Kjeldahl nitrogen (TKN) in slurry was also similar ($P=0.682$). However, RSM-based diets significantly reduced NH_3 emissions compared to SBM-based diets, with lower NH_3 losses per kg slurry (110.4 vs. 155.2 mg; $P=0.001$), per kg diet (112.5 vs. 188.6 mg; $P=0.006$), and per animal per day (189.7 vs. 289.6 mg; $P=0.017$). NH_3-N losses relative to dietary TKN were also lower in RSM-fed pigs (4.52 vs. 6.47 g/kg; $P=0.032$). These reductions were associated with lower urinary N excretion in RSM-fed pigs compared with SBM-fed pigs (3.07 vs. 4.53 g/kg diet; $P<0.001$) and a higher fecal/urinary N ratio (1.05 vs. 0.59; $P<0.001$), indicating a shift from urinary to fecal N excretion, thereby mitigating volatile losses. Additionally, RSM-fed pigs excreted slurry with a lower pH than SBM-fed pigs (7.21 vs. 7.89; $P\leq0.001$), further reducing NH_3 volatilization. SFM-based diets showed intermediate NH_3 emissions and a numerically lower BMP value, with CH_4 production of 176.1, 194.0, and 206.8 mL CH_4/g OM for SFM, RSM, and SBM, respectively ($P=0.303$). These results highlight the potential of RSM as a dietary strategy to mitigate NH_3 emissions by decreasing urinary N losses and slurry pH, thereby improving the environmental footprint of pig production. Methane emissions, however, were not significantly affected by the dietary replacement of SBM with RSM or SFM.

References

Beccaccia, A.F., Calvet, S., Cerisuelo, A., Ferrer, P., García Rebollar, P., De Blas, J.C., 2015. Animal Feed Science and Technology 208, 158–169. https://doi.org/10.1016/j.anifeedsci.2015.07.008

Martín González, L., Colturato, L.F.F., Font, X., Vicent, T., 2010. Waste Management 30, 1854–1859. https://doi.org/10.1016/j.wasman.2010.03.029

Effects of pig allocation and feeding strategy on growth performance of growing-finishing pigs

A.J.M. Jansman[1], M.H.A. van Helvoort[2], N. Stevens[2], J. Camp Montoro[3], J. Htoo[4], R. de Mol[1], E. M. A. M. Bruininx[2]
[1]Wageningen Livestock Research, Wageningen, Netherlands. [2]de Heus Animal Nutrition B.V., Ede, Netherlands. [3]PIC, Barcelona, Spain. [4]Evonik Operations GmbH, Nutrition & Care, Hanau-Wolfgang, Germany (alfons.jansman@wur.nl)

To increase N-use efficiency in pigs, refined feeding strategies should be developed and implemented. The aim of the present experiment was to evaluate the effects of: 1. animal allocation strategy at pen level based on birth weight (BiW) and breeding value (BV) for average daily gain (ADG), and 2. application of a 3-phase or a precision feeding system for standardized ileal digestible (SID) Lys on a weekly basis on the growth performance and N-use efficiency in growing-finishing (GF) pigs (25-125 kg body weight (BW)). The trial was conducted with 384 GF gilts (PIC 800 Duroc boar x TN 70 sow) in a 2 x 2 x 2 factorial arrangement with 8 pens (6 animals each) per treatment. Pigs were fed a fixed diet per phase (4 weeks) or provided a different diet per week with an adjusted concentration of SID Lys by mixing a low and a high SID Lys diet to match the assumed requirement for SID Lys using a factorial algorithm to predict the SID Lys requirement over a period of 1 week, based on the forecasted average daily feed intake (ADFI) and ADG of animals per pen in the following week. BW at the start ($P<0.01$) and at delivery ($P<0.001$), ADG (1188 vs 1127 g/d; $P<0.001$) and ADFI (2.78 vs 2.66 kg/d; $P<0.01$) were higher in high BiW than in low BiW animals. FCR was not affected by BiW. Pigs with a high BV for ADG were heavier upon delivery and had a higher ADG (1185 vs 1129 g/d; $P<0.001$), and ADFI (2.79 vs 2.65 kg/d; $P<0.01$) than pigs with a low BV for ADG. FCR did not differ in animals with a low or high BV for ADG. Precision fed pigs tended ($P=0.09$) to have a higher BW at delivery compared to phase fed animals. However, ADG, ADFI and FCR did not differ between 3-phase or precision fed pigs. Calculated nitrogen use efficiency was improved by precision feeding compared to 3-phase feeding (51.0 vs 49.9%; $P<0.01$). The higher growth performance of pigs with a high BiW and high BV for ADG compared to pigs with a low BiW and low BV for ADG indicates that BiW and the BV for growth performance (ADG) affect absolute body protein retention but do not affect N-use efficiency in pigs. Feeding pigs according to a 3-phase feeding system or to a weekly precision feeding system based on supply of SID Lys at pen level, does not affect growth performance, but increases the efficiency of utilization of nitrogen. Further refining the algorithms for forecasting feed intake and body weight gain of pigs at pen level could help to improve precision feeding systems and further increase N-use efficiency.

Funding

This research was carried out by Wageningen Livestock Research as part of the Public Private Partnership “Precision feeding of pigs for further reducing emissions and improving animal health (PFP 2.0)” (LWV19050) and funded by the Ministry of Agriculture, Fisheries, Food Security and Nature (LVVN), De Heus, Hotraco, PIC and Evonik.

Dietary crude protein reduction in poultry diets allows to decarbonize poultry meat production, comparison between broilers, ducks and turkeys

M. Durand, C. Raybaud, P. Moquet, W. Lambert
Mixscience, Bruz, France (william.lambert@mixscience.eu)

Feed accounts for around 75 % of the environmental impact of the poultry meat production, regardless of the production method. This study aimed to compile recently conducted poultry experiments with the same goal and methodology: studying the impact of reduced dietary crude protein (CP) levels on growth and feed intake performance as well as measuring the benefits in terms of carbon footprint. Five different trials were compiled and compared across broiler genetic lines (Ross 308, JA 787, S757N) and segments (broiler, turkey, duck). Dietary CP reduction was achieved in all segments by gradually replacing imported soybean meal by cereals and feed-grade amino acids (AA). Dietary digestible Lysine (dLys) supply was constant for all treatments within each experiment, and dietary AA supply of other essential AA was controlled at least at the assumed requirement. With this protocol, it was possible to reduce dietary CP in diet formulation, expressed as mg dLys/ point of dietary CP as low as: 52 to 61, 49 to 58, 45 to 54, 47 to 57, and 56 to 62, for Ross 308 broiler, JA 787 broiler, S757N broiler, duck, and turkey, respectively. Based on growth performance and meat yields, the lowest reachable levels of dietary CP reduction with dietary AA, without impacting performance were: under 55 mg dLys/pt CP for JA 787 and S757N broilers, under 60 mg mg dLys/pt CP for Ross 308 broilers and duck and under 65 mg dLys/pt CP for turkey. It is possible to reduce dietary CP in all segments and species, but the level of nutrient density reachable differed within the broiler segment (Ross vs JA 787 vs S757N), and between broilers, ducks and turkeys. One explanation could be found in nitrogen use efficiency (improve by 2 to 7 pts) and in the diet profil of protein-rich feedstuffs. By reducing dietary CP, reducing imported soybean meal and implementing MatriCIEL calculations, it was also possible to reduce carbon footprint (in kg $CO2_{eq}$/kg meat) by 2-8 % and nitrogen excretion between 7 to 32 %.

Circular ingredients in diets of growing pigs: effects on nutrient digestibility, nitrogen retention and digesta retention time

M.O. Wellington[1], P. Bikker[2], A.J.M. Jansman[2]
[1]Trouw Nutrition R&D, Boxmeer, Netherlands. [2]Wageningen University and Research, Wageningen, Netherlands (michael.wellington@trouwnutrition.com)

Higher amounts and various combinations of alternative ingredients or co-products may improve circularity of swine diets. These relatively high-fibre ingredients may have implications for nutrient digestibility. The aim of the present study was to determine the effects of circular diets differing in ingredient composition on the kinetics of nutrient digestibility, and nitrogen (N) retention in growing pigs. We hypothesized that N-retention would not differ among the circular diets, but nutrient digestibility and N-utilisation efficiency would be reduced by the inclusion of circular ingredients. Five experimental diets with circular ingredients were tested. The control diet (T1) was formulated as a wheat, soybean meal (SBM) diet, while in T2 and T3, 100% of the wheat in T1 was replaced with bakery products (chocomix, bread, and biscuit meal) and cereal co-products (maize DDGS, wheat gluten and maize feed meal) respectively. In T4 and T5, 100% of the SBM in T1 was replaced with legume seeds (peas, faba bean, and lupins) and oil seed meals (rapeseed and sunflower meal) respectively. A total of 40 individually housed barrows (8 pigs/treatment; BW = 31.5 ± 2.2 kg), were used in a 5-day N-balance experiment, followed by a two-day frequent feeding to steady state for digesta kinetics measurements. The apparent ileal/total tract digestibility (AID/ATTD) of nutrients, and volatile fatty acid (VFA) in digesta were determined. Acid-insoluble ash was used as marker for determining nutrient digestibility. No differences ($P>0.05$) were observed for N intake, faecal and urinary N, and N-retention. Absolute N-retention varied ($P>0.05$; T3;23.3 g/d vs. T4; 26.9 g/d), while N-retention efficiency, varied (T3;72.6 vs. T4;78.1 %) but not significantly different ($P>0.05$). The AID of Thr was lowest in T3 and T5 (~70%) compared to the others (~78%). The ATTD of N was not significant ($P>0.05$) but varied between diets (e.g., T3;78% vs T2;85%). In the cecum and colon, T4 had the highest propionic acid concentration ($P<0.05$), but with no effect on other VFAs. The mean retention time (MRT) in the stomach digesta solids was higher than liquid fraction (3.35 h vs. 1.54 h; $P<0.05$). The MRT in the proximal and distal small intestine between digesta solids and liquid was not different ($P>0.05$). In the stomach liquid fraction, there was a tendency ($P=0.09$) for a higher MRT in T3 (2.64 h) compared to T5 (1.20 h). In the cecum, there was a higher MRT ($P<0.05$) of the liquid fraction in T4 (3.23 h) compared to T2 (1.63 h), but not different from T1 (2.74 h). Including circular ingredients did not affect N-retention efficiency, contrary to our hypothesis. In conclusion, replacing wheat and soybean meal with circular ingredients may be possible, offering a promise for the sustainability and cost-effectiveness of swine diets.

Funding

This research was conducted within the public private partnership “Feed4Foodure-III; Sustainable animal nutrition in circular agri-food systems” (TKI-Agrifood LWV20203), partially funded by the Ministry of Agriculture, Fisheries, Food Security and Nature (project BO-55-001-015), and partly by “Vereniging Diervoederonderzoek Nederland”.

Identification of cow-related parameters for assessment of protein feeding management on dairy farms

N. De Neve, T. Van den Bossche, L. Vandaele, K. Goossens
Flanders Research Institute for Agriculture, Fisheries and Food (ILVO), Melle, Belgium (Karen.Goossens@ilvo.vlaanderen.be)

The Flemish dairy cattle sector is required to reduce ammonia emissions by 25% by 2030. Low protein feeding is an effective measure to reduce NH_3 emissions, but a method to monitor the crude protein (CP) levels in the ration is lacking. A trial was conducted in the ILVO dairy cattle barn to identify cow-related parameters correlating with the dietary CP intake. Thirty-six Holstein cows (15 primiparous, 21 multiparous, 2±1.2 lactations, 671±80.8 kg BW, 111±63 DIM, 39.3±6.35 kg milk/day) were fed diets containing 150, 160, 160 with non-protein nitrogen (NPN, urea) or 170 g/kg CP in a latin-square design with four experimental periods. All diets met the MP requirements, were isocaloric and formulated as total mixed ration (TMR). Experimental periods lasted three weeks with collection of milk, blood, urine, feces, saliva and hair samples in the final week. Results were analyzed at herd level using a mixed model with diet and DIM as fixed factors and cow as random factor. Dry matter intake tended to be higher for the 160 diet compared to the 150 diet with intermediate intake for the 160NPN and 170 diet. The intake of true protein digested in the small intestine (DVE) and rumen degradable protein (RDP) varied according to the dietary protein level (150<160=160NPN<170). Milk yield, fat-and-protein-corrected milk yield, milk fat yield or milk protein yield did not differ significantly. Milk urea and urinary urea concentrations were affected by CP and RDP intake (150<160<160NPN<170), while urinary nitrogen excretion, urinary urea excretion and serum urea/creatinine ratio varied according to the CP level (150<160=160NPN<170). Urinary nitrogen concentration increased with increasing CP level but was also influenced by RDP intake. The nitrogen isotopic abundance in hair, urea content in saliva and fecal nitrogen excretion per day did not correlate with the dietary CP level. Fecal ammonia nitrogen and fecal nitrogen content were different between low and high protein diets (150<170) but showed variable results for intermediate diets. Stepwise regression with leave-one-out cross-validation identified four combinations of two parameters for estimating dietary CP content. The most accurate model included bulk milk urea-creatinine-ratio and urinary nitrogen excretion per day (RMSE = 2.87 g/kg DM), followed by a model of bulk milk urea-creatinine ratio and bulk milk protein content (RMSE = 3.05 g/kg DM). A third model consisted of urinary urea excretion per day and urinary nitrogen content (RMSE = 3.09 g/kg DM), followed by a model with bulk milk urea and serum urea/creatinine ratio (RMSE = 3.23 g/kg DM). In experimental conditions, CP content can be estimated with reasonable accuracy using cow-related parameters. Further validation is needed under practical farm conditions to assess the robustness of these prediction models.

Funding

This research was funded by the government agency Flanders Innovation and Entrepreneurship (VLAIO, Schaerbeek, Belgium; HBC.2022.0931).

Biomarkers-assisted precision feeding in beef cattle: performance and metabolic impacts

E. Jorge-Smeding[1], H. Aboshady[2,1], S. Taussat[3,4], S. Dumerchat[5], B. Deroche[6], M. Legris[7], J-J. Bertron[6], G. Cantalapiedra-Hijar[1]

[1]Université Clermont Auvergne, INRAE, VetAgro Sup, UMR Herbivores, 63122 Saint-Genès-Champanelle, France. [2]Department of Animal Production, Faculty of Agriculture, Cairo University, Giza, Egypt. [3]INRAE, AgroParisTech, GABI, Université Paris-Saclay, 78350 Jouy-en-Josas, France. [4]Allice, 149 Rue de Bercy, 75595 Paris Cedex 12, France. [5]Chambre d'Agriculture des Pays de la Loire, Ferme des Etablières, 85000 La Roche Sur Yon, France. [6]Institut de l'Elevage, Services Productions de Viandes, 49071 Beaucouzé, France. [7]Institut de l'Elevage, Services Datastat, 75595 Paris, France (gonzalo.cantalapiedra@inrae.fr)

Between-animal variations in feed efficiency imply different nutritional requirements for individuals sharing the same pen. This study investigated the effects of precision feeding, assisted by biomarkers of residual feed intake (RFI), on the performance and metabolism of young bulls. The RFI ranking of 119 Charolais young bulls was predicted based on plasma metabolites (Aboshady et al.,2024). Seventy-two animals, selected from lowest (LRFI), intermediate (IRFI) and highest RFI (HRFI) ranks, were assigned to three diets with contrasting forage-to-concentrate ratios: 50:50, 65:35 and 80:20. The animals were housed in three pens of 24 animals each, sharing the same diet. Three farms were tested: Classic (n = 24; where HRFI and LRFI animals were all fed the 65:25 diet); Precision (n = 24; where HRFI was fed the 80:20 diet, while the LRFI was fed the 50:50 diet) and Control (n = 24; where IRFI were fed either the 50:50 or the 80:20 diet) over 174 days until slaughter. The fattening period included two consecutive feed efficiency tests, during which daily dry matter intake (DMI), fortnightly live weight (LW), average daily gain (ADG), feed conversion efficiency (FCE), and RFI were measured. Plasma metabolites were assessed at the end of the first test, and carcass traits were determined at slaughter. Performance data was analysed considering the farm, the test, and its interaction as fixed effects. The time was the repeated factor, and the animal nested in the Farm the random effect; while the 1st order autoregressive was considered as the covariance structure. Plasma metabolites and carcass traits were analysed considering the farm as the only fixed effect. Both models considered initial LW as a covariate. The DMI was lower in Precision vs Classic (9.8 vs. 10.5 kg/day; $P = 0.01$), with intermediate values for Control (10.1 kg/day). RFI was the highest in the Classic farm ($P < 0.01$). No significant effects were observed for ADG, FCE or carcass weight. Carcass fat was higher in Control vs. Classic (2.2 vs. 1.8 %, $P < 0.01$). Plasma β-hydroxybutyrate was higher in Control vs. Precision (0.250 vs. 0.210 µM; $P = 0.03$). No significant effects were detected for glucose, non-esterified fatty acids, urea or creatinine. In conclusion, biomarkers-assisted precision feeding decreased the overall feed intake compared to a homogenous diet for all animals. However, no significant effects were observed on ADG and FCE, while minor effects on the metabolic status of young bulls were noted.

Reference

Aboshady, H.M., Jorge Smeding, E., Taussat, S., Cantalapiedra Hijar, G., 2024. Animal 18, 101354. https://doi.org/10.1016/j.animal.2024.101354

Funding

APISGENE (Nutrimarkers project)

Effects of restricted feeding on enteric methane production and energy and nitrogen balance in growing beef × dairy steers

M.S. Grant[1], L.G. Canterbury[1], C.W. Dornbach[1], K.D. Childress[1], P.R. Broadway[2], N.C. Burdick Sanchez[2], B.W. Neville[3], M.L. Galyean[4], K.E. Hales[1]
[1]Department of Animal and Food Sciences, Texas Tech University, Lubbock, TX, USA. [2]USDA-ARS, Livestock Issues Research Unit, Lubbock, TX, USA. [3]USDA-ARS, U.S. Meat Animal Research Center, Clay Center, NE, USA. [4]Department of Veterinary Sciences, Texas Tech University, Lubbock, TX, USA (kristin.hales@ttu.edu)

Dry matter intake (DMI) is a strong predictor of enteric methane production in beef cattle (Galyean and Hales, 2024). Limit feeding is a management strategy that can increase gain efficiency in growing and finishing beef cattle. Our objective was to evaluate the effects of restricted DMI on enteric methane production and energy and nitrogen balance in beef × dairy steers. Seven beef × dairy steers (initial BW = 306 ± 18.5 kg) were used in a repeated (one complete and one incomplete) Latin square design with 4 periods. Treatments included feed offered to slick bunk management to target no residual feed at 0730 h (100R) and 4, 8, or 12 % restriction of slick bunk management (96R, 92R, and 88R, respectively). Experimental periods consisted of a 14-d diet adaptation, 7 d of estimation of DMI and total fecal and urine collections, and a 24-h gas exchange collection using indirect respiration calorimeter headboxes. Data were analyzed as a repeated Latin square design with using the MIXED procedure of SAS version 9.4 (SAS Inst. Inc., Cary, NC, USA) and the Kenward Rogers degrees of freedom adjustment was used. The model contained fixed effects of treatment and period and random effects of steer and steer within square. The LSMEANS statement was used to calculate treatment means and significance was declared at $P \leq 0.05$ and tendencies at $0.05 \leq P \leq 0.10$. Dry matter intake did not differ among treatments ($P=0.84$) and averaged 7.8, 7.7, 7.7, and 7.8 kg/d for 100R, 96R, 92R, and 88R, respectively (SEM=0.30). Lack of differences in DMI resulted from restrictions being applied within periods of the Latin square and occasional feed refusals on a given day within periods. Consequently, intake energy (IE) and apparent DM digestibility did not differ among treatments ($P \geq 0.71$). Fecal energy loss, digestible energy (DE), and urinary energy loss also did not differ among treatments ($P \geq 0.55$) nor did methane energy loss and methane loss as a proportion of IE ($P \geq 0.81$). Metabolizable energy (ME), ME as a proportion of IE, and ME as a proportion of DE did not differ among treatments ($P \geq 0.60$). Likewise, heat production (HP), HP as a proportion of IE, recovered energy (RE), and RE as a proportion of IE did not differ among treatments ($P \geq 0.87$). Furthermore, nitrogen intake and apparent nitrogen digestibility fecal and urinary N excretion, and fecal and urinary N excretion as a proportion of total N intake did not differ among treatments ($P \geq 0.30$). As a result, retained N and retained N as a proportion of N intake also did not differ among treatments ($P \geq 0.47$). Overall, no differences in energy or N balance were detected, likely because of the lack of differences in DMI and IE.

Reference

Galyean, M.L., Hales, K.E., 2024. Methane 3, 1–11. https://doi.org/10.3390/methane3010001

Funding

Research was supported by the National Cattlemen's Beef Association, a contractor to the Beef Checkoff.

Effects of guanidinoacetic acid supplementation on performance, carcass traits and meat quality of late growing pigs under heat stress

J.K. Htoo[1], A. Morales[2], N. Arce[2], S. Hernánde[2], L. Camacho[2], E. Avelar[2], M. Cervantes[2]
[1]Evonik Operations GmbH, Hanau-Wolfgang, Germany. [2]ICA-Universidad Autónoma de Baja California, Mexicali, Mexico (john.htoo@evonik.com)

Exposure to heat stress (HS) can reduce feed intake, growth performance (Morales et al., 2018) and impair carcass quality of pigs (Mun et al., 2022). A 36-day study was conducted to determine the effects of supplementing guanidinoacetic acid (GAA) under heat stress (up to 38°C) environment on performance, carcass characteristics and meat quality of late growing pigs. Thirty-two mixed-sexed pigs (Landrace × Hampshire × Duroc; initial body weight (BW) of 44.4 ± 4.8 kg) were assigned to 2 treatments in 2 blocks having 8 pen replicates (2 pigs per pen) per treatment. Pigs had free access to water and feed. The treatments were 1) control diet (CON) and 2) 0.09% GAA (GAA) was added to CON replacing corn starch. Diets were based on corn, soybean meal and amino acids (AA) which met AA requirements for 45 to 75 kg pigs (0.94% SID Lys; 10.40 MJ/kg net energy). The ambient room temperature was recorded. Blood samples were collected from the jugular vein at 2 hours (h) after the last meal to measure the serum concentration of superoxide dismutase (SOD). At slaughter, 16 pigs (8 pigs/treatment) were selected for carcass assessment. Samples of longissimus dorsi (LD) muscle were used for meat quality assessments. The pH, muscle color and drip loss were measured using a pH meter at 24 h after slaughter. Data were analyzed by ANOVA using Proc GLM of SAS. The model included block and initial BW as a covariate. Sex effect was not significant and not included in the model. Final body weight (76.98 vs. 77.26 kg; $P = 0.808$) and average daily gain (ADG; 0.896 vs. 0.904 kg; $P = 0.792$) were not different among CON and GAA group. Compared with CON, GAA supplementation reduced daily feed intake (1.93 vs. 1.79 kg/d; $P = 0.024$) and improved FCR (2.18 vs. 2.00; $P = 0.009$). In comparison to CON, GAA supplementation did not affect ($P > 0.05$) chilled carcass weight (53.55 vs. 53.53 kg), chilled carcass yield (70.2 vs. 71.4 %), loin eye area, (39.4 vs. 44.6 cm^2), 24 h chilled carcass pH (6.31 vs. 6.27), backfat thickness (13.9 vs. 14.4 mm) and drip loss after 24 h (3.02 vs. 3.53%). The color scores (L, Lightness; a, red intensity; b, yellow intensity) of longissimus muscle were not affected ($P < 0.05$) by GAA supplementation. However, GAA supplementation tended to increase serum concentrations of superoxide dismutase (2.64 vs. 2.26 U/ml; $P = 0.080$) indicating an enhanced anti-oxidant capacity of GAA pigs. These results indicate that supplementation with GAA (0.09%) in late growing pig diet improved FCR and may increase anti-oxidant capacity of the pigs.

References

Mun, H.S., Rathnayake, D., Dilawar, M.A., Jeong, M.G., Yang, C.J., 2022. Journal of Applied Animal Research 50, 103–108. https://doi.org/10.1080/09712119.2022.2032084
Morales, A., Chávez, M., Vásquez, N., Htoo, J.K., Buenabad, L., Espinoza, S., Cervantes, M., 2018. Journal of Animal Science 96, 1419–1429. https://doi.org/10.1093/jas/sky044

Funding

This research was funded by Evonik Operations GmbH.

Hepatic fatty-acid driven respiration of dairy cows with contrasting environmental conditions during supplementation in pasture-based production systems

M. Carriquiry, G. Cañibe, M.M. García-Roche
Facultad de Agronomía, UdelaR, Montevideo, Uruguay (mcarriquiry@fagro.edu.uy)

Mitochondria are important modulators of the energetic response and adaptation to stress (Skibiel, 2023). Particularly, it has been proposed that heat stress impaired mitochondrial function, inducing oxidative stress and reducing fatty acid oxidation. Thus, spring-calved multiparous Holstein cows (n=18, 588±26 kg body weight, 2.8±0.1 body condition score, mean±SD) in mid-lactation (130±13 days in milk, DIM) were used in a randomized block design to evaluate the effect of environmental exposure during summer on hepatic fatty-acid driven respiration. Cows grazed a mixed pasture (one session from 19:00 to 3:00 h; 35 kg DM/d of herbage allowance), and were supplemented with a total mixed ration (TMR) offered after the morning milking (9.5 kg DM/d, 30:70 forage:concentrate) in a compost barn (with fan and soakers; CB; n=9) or in an open pen (OP; n=9). Blood samples and liver biopsies were collected at 135 and 185±13 DIM; 4 days before both biopsy collections temperature humidity index was > 70 for at least 9 h per day. Mitochondrial fatty acid respiration was assessed in the cryopreserved biopsies measuring oxygen consumption rates in a high-resolution respirometer (Oxytherm+R; Hansatech Instruments Ltd., UK) sustained by specific substrates (García-Roche et al., 2023). Data were assessed as repeated measures with a model including treatment, DIM and their interaction as fixed effects; means (±SE) were considered to differ when $P<0.05$. Average body temperature measured using surface sensors (Thermochron, Maxim Integrated Inc., MA, USA), during summer (130 and 185±13 days in milk) was greater for OP than for CB cows (37.0 vs. 37.6±0.04°C), particularly during TMR supplementation when cows were exposed differently to the environmental conditions (6:00 to 16:00 h). However, neither energy corrected milk yield (29.5 vs. 29.8±1.2 kg/d) nor plasma non-esterified fatty acid (0.37 vs. 0.41±0.07 mmol/L) or triglycerides (27.5 vs. 1.8±1.8 mg/d) concentrations differed between CB and OP cows. Mitochondrial fatty acid-driven respiration parameters were not affected by DIM or the interaction of treatment by DIM. However, on average, state 4 (palmitoyl-CoA) and oligomycin-resistant sensitive respiration (ATP-independent respiration) were greater for OP than CB cows (0.38 vs. 0.29±0.06 and 0.26 vs. 0.14±0.04, respectively) while state 3 (when ADP was added) and oligomycin-sensitive respiration (oxygen consumption linked to ADP phosphorylation) did not differ between treatments (0.38 vs. 0.35±0.08 and 0.16 vs. 0.20±0.05, for OP and CB, respectively). In addition, leak control ratio (protons returning to matrix independently of ATP synthase) was greater for OP than CB (0.64 vs. 0.49±0.04). Our results showed reduced environmental exposure (CB) during heat-stress conditions improved mitochondrial function, leading to a more efficient oxidation of fatty acids in mid-lactation dairy cows.

References

Skibiel, A.L., 2023. JDS Communications 5, 247–252. https://doi.org/10.3168/jdsc.2023-0432
García Roche, M., Talmón, D., Cañibe, G., Astessiano, A.L., Mendoza, A., Cassina, A., Quijano, C., Carriquiry, M., 2023. PLOS ONE 18, e0290551. https://doi.org/10.1371/journal.pone.0290551

Funding

The project was funded by ANII INNOVAGRO 2018: FSA_1_2018_1_152220 to M. Carriquiry and A. Cassina.

Strategies to improve the nitrogen use efficiency of grasslands and protein quality of grass silage

T. Van den Bossche[1], J.L. De Boever[1], G. Haesaert[2], M. Cougnon[1], S. Debevere[3], L. Vandaele[1], K. Goossens[1]
[1]ILVO, 9090 Merelbeke-Melle, Belgium. [2]Gent University, 9000 Gent, Belgium. [3]Inagro, 8800 Rumbeke-Beitem, Belgium (tine.vandenbossche@ilvo.vlaanderen.be)

Grasslands are the primary forage source for ruminants, and as global food demand rises, optimizing their efficiency and quality is essential (Lüscher et al., 2014). New grass varieties and technologies improved grassland yields and quality, but the highest improvement was achieved through fertilization. However, excessive nitrogen (N) fertilization is unsustainable, as nearly half of the applied N is lost through volatilization, runoff, or leaching. This loss contributes to acidification, eutrophication, and biodiversity loss, while degrading air, soil, and water quality (Martinez-Dalmau et al., 2021). To maintain profitability and comply with national legal N limits, farmers must enhance nitrogen use efficiency (NUE) and improve grass protein quality. This study evaluated grassland management on 53 fields across 10 dairy farms in Flanders to identify best practices for optimizing NUE and grass silage protein quality. Data were collected over one year (2020). Soil and manure samples were analyzed before the first fertilization to assess plant-available N. Pre-wilted grass yield was estimated. Additionally, pre-wilted grass samples (n=65) were collected before ensiling, and corresponding grass silage samples (n=41) were analyzed. Clover presence was determined based on seed mixture composition. Annual NUE was calculated where possible (n=28). The objective was to identify best practices for optimizing NUE and improving grass silage protein quality. Fields with grass–clover mixtures exhibited higher NUE, due to increased dry matter (DM) yield combined with significantly lower N fertilizer input. While monocultures of highly productive grasses generally achieve higher DM yields under high N fertilization, our study found that within the applied fertilizer range, grass–clover fields tended to produce higher DM yields. This was likely due to a positive diversity effect and the warm, dry conditions of the trial year. Clover-containing fields (7%–40% clover) required significantly less N fertilizer than pure grassland fields to achieve comparable yields. Grass–clover fields reached the mean DM yield (11 t/ha/year) with 71% less N fertilizer (75 vs. 265 kg/ha). Similarly, the mean digestible protein yield (DVE: 759 kg/ha/year) and N yield (272 kg/ha/year) were obtained with 60% (100 vs. 250 kg/ha) and 44% (110 vs. 250 kg/ha) less N fertilizer, respectively. Protein quality in grass silage was positively correlated with organic matter digestibility, sugar content, and DM content of pre-wilted grass, whereas crude ash content had a negative impact. These relationships suggest that specific management practices, such as mowing at an early growth stage, during sunny afternoons, after sufficient pre-wilting, and minimizing soil contamination, enhance protein quality. These findings demonstrate that incorporating clover into grassland systems enhances NUE and reduces fertilizer dependency while maintaining productivity, provided that N fertilization is properly adjusted. Additionally, practical management strategies can further optimize grass silage protein quality.

References

Lüscher, A., Mueller-Harvey, I., Soussana, J.F., Rees, R.M., Peyraud, J.L., 2014. Grass and Forage Science 69, 206-228. https://doi.org/10.1111/gfs.12124
Martínez-Dalmau, J., Berbel, J., Ordóñez-Fernández, R., 2021. Sustainability 13, 5625. https://doi.org/10.3390/su13105625

Funding

Agentschap Innoveren en Ondernemen, Grant/Award Number: HBC.2018.2222

Inclusion of lemon leaves and maralfalfa as alternative forage to alfalfa and its effect on energy balance, milk performance and methane emissions in dairy goats

C. Fernández[1], M. Lachica[2]
[1]Universitat Politecnica de Valencia, Valencia, Spain. [2]Estación Experimental del Zaidin, Granada, Spain (cjfernandez@dca.upv.es)

The objective of this experiment was to study the effects of replacing alfalfa (*Medicago sativa*) with maralfalfa (*Pennisetum sp.*) and lemon leaves (*Citrus limonum*) on energy balance, milk performance and methane (CH_4) emission in dairy goats. Twelve Murciano-Granadina dairy goats at mid lactation weighting 46.1 ± 0.57 kg were used in a uncomplete crossover design. Each goat received the 3 treatments in 3 periods. One group of 4 goats was fed a mixed ration with alfalfa as forage (A diet), other with maralfalfa (M diet) and the third group with lemon leaves (L diet). The diet had a forage concentrate ratio 45/55 and the concentrate was a pelletized commercial compound feed. Diets were formulated to be isoenergetic and isoproteic. The goats were allocated to individual metabolism cages. After 10-d of adaptation, feed intake, total fecal, urine output and milk yield were recorded daily over a 5-d period. Then, gas exchange measurements were recorded individually by a mobile open-circuit indirect calorimetry system using a head box for 2 more days. The data were analyzed by mixed model and differences were significant when $P<0.05$. Gross energy intake was different ($P < 0.05$) among diets being greater in A followed by M and L. Differences were observed in energy losses in feces, being greater in M diet than others. Tropical C4 grasses (as maralfalfa) lead to a higher rate and degree of deposition of lignin in plant tissue, a factor that may reduce voluntary intake and digestibility (Wilson, 1994). So, metabolizable energy intake was lower in M (991 kJ/kg of $BW^{0.75}$) and higher in A and L (1,094 kJ/kg of $BW^{0.75}$, on average). No differences were observed in energy deposited in milk (342 kJ/kg of $BW^{0.75}$, on average) and heat production (643 kJ/kg of $BW^{0.75}$, on average). Therefore, energy balance was greater in A, followed by L and M ($P < 0.05$). No differences were observed for milk yield, fat, protein and lactose composition (1.8 kg/d, 5%, 4.3% and 4.5%, respectively). Differences was observed for CH_4 emissions ($P < 0.05$) and when it was expressed over milk yield, greater emission was observed for M, followed by A and L (16, 10 and 8 g CH_4/kg milk, respectively). Palmquist and Jenkins (1980) indicated that dietary lipid (as lemon leaves) is more likely to inhibit fiber degradability with a concomitant reduction in fermentation and, therefore, CH_4 emissions. Use of tropical grasses, such as maralfalfa, in temperate climates could be a strategy to incorporate forage to feed the flock by farmers, because milk chemical composition did not change. Lactating goats could utilize lemon leaves as forage without detrimental effect on milk performance. Data suggest that incorporating lemon leaves into lactating goat diets reduced CH_4 emissions.

References

Palmquist, D.L., Jenkins, T.C., 1980. Journal of Dairy Science 63, 1-14. https://doi.org/10.3168/jds.S0022-0302(80)82881-5
Wilson, J.R., 1994. Journal of Agricultural Science 122, 173-182. https://doi.org/10.1017/S0021859600087347

In vitro ruminal degradation of dihalomethanes

M. Thorsteinsson[1], M. Rønn[2], M.O. Nielsen[1]
[1]Aarhus University, Tjele, Denmark. [2]SEGES Innovation, 8200, Denmark (mitho@anivet.au.dk)

Volatile halogenated compounds are known to possess anti-methanogenic properties with most in vivo studies focusing on trihalomethanes. However, concerns can be raised regarding the use of these compounds as methane-mitigating additives due to the transfer of halogenated metabolites into milk which may pose a threat to consumer health. Transfer of halogens originating from halogenated anti-methanogenic feed additives into milk can, at least in theory, be reduced by reducing the number of halogen atoms on the molecule. Halogenated compounds are often quickly dehalogenated by methanogens (Glasson et al., 2022), which can affect the methane-mitigation potential of the halogenated compounds. This study aimed to investigate the rumen degradation kinetics of three dihalomethanes, namely bromoiodomethane (BIM), chloroiodomethane (CIM), and diiodomethane (DIM), as these compounds have demonstrated great anti-methanogenic properties in vitro with reductions in methane production ranging from 58% for CIM and <90% for BIM and DIM (Thorsteinsson and Nielsen, 2025). The compounds were dissolved in 99% ethanol, reaching a final concentration of 200 ng/mL for BIM and CIM and 270 ng/mL for DIM. The solutions (0.1 mL) were added into bottles with buffered rumen fluid and incubated at 38.5°C for 0, 5, 10, 15, 20, 30, 40, 60, 120, 240, 360, 1440, 2160, and 2880 min in a controlled incubator shaker. An ANKOM pressure sensor module (ANKOM Technology, Macedon, NY, USA) was mounted on each bottle, allowing venting of the bottles during incubation. By the end of the incubation period, two replicate samples of 1 mL were collected from each bottle and immediately treated with 1 mL of methanol to stop the fermentation instantly. Concentrations of dihalomethanes in rumen fluid were measured using a GC-MS/MS quantification method. Data was statistically analysed using a linear model procedure of R. All dihalomethanes were metabolized by the rumen microbiota with half-lives (T½) being 255, 589, and 691 min for CIM, DIM, and BIM, respectively. The concentration of CIM was below the detection level (10 ng/mL) after 1440 min i.e. 24h, while concentrations of BIM and DIM were below the detection level (2 ng/mL for both compounds) after 2160 min i.e. 36h. In conclusion, half-lives of the dihalomethanes can explain the reported greater methane-mitigating potential for BIM and DIM compared with CIM.

References

Glasson, C.R.K., Kinley, R.D., de Nys, R., King, N., Adams, S.L., Packer, M.A., Svenson, J., Eason, C.T., Magnusson, M., 2022. Algal Research 64, 102673. https://doi.org/10.1016/j.algal.2022.102673

Thorsteinsson, M., Nielsen, M.O., 2025. JDS Communications 6, 329-332. https://doi.org/10.3168/jdsc.2024-0700

Funding

The study was funded by the National Green Development and Demonstration Program (GUDP grant no.: 34009–22-2093), Denmark.

Can carrots replace maize in a dairy sheep diet? A Rusitec study

A. Díaz[1,2], N. Merino[1,2], C. Saro[1,2], I. Mateos[1,2], M.J. Ranilla[1,2], V. Rodríguez[1]
[1]Universidad de León. Dpto. Producción Animal, León, Spain. [2]Instituto de Ganadería de Montaña. Finca Marzanas, Grulleros, Spain (mjrang@unileon.es)

Carrots are the third most frequently cultivated vegetables in the European Union but a great amount of them is discarded after harvesting due to sizing problem or cosmetic reasons. These discarded carrots could be included as feedstuff in ruminant diets, contributing to minimize the environmental impact of agriculture and food industry, because residues would otherwise generate waste or pollutants, thus increasing the carbon footprint. The aim of this study was to evaluate the effect of replacing maize by carrots in a conventional dairy sheep diet on rumen fermentation characteristics in Rusitec fermenters. Two 1:1 forage:concentrate ratio diets were formulated: a control diet (CON) and another with 16 % of carrots (CAR) totally replacing maize in the control diet. Diets were formulated to have similar crude protein (CP; 16.6 %) and neutral detergent fibre (NDF; 27.4 %) contents. Two identical 13-day incubation runs were carried out independently using 4 Rusitec fermenters, and each of the two diets (20 g dry matter/day) was assigned randomly within each experimental run so that two fermenters received one of them. Ruminal contents (solid and liquid) of four rumen-cannulated non-lactating Assaf sheep (56.3 ± 1.56 kg of body weight) were used to inoculate the Rusitec fermenters. After 7-d of diet adaptation, diet disappearance and fermentation parameters were assessed in four consecutive days. Data were analysed as a mixed model with repeated measures using the PROC MIXED of the Statistical Analysis System (SAS Institute Inc., Cary, NC, USA). The statistical model used included the diet, incubation run, time and diet x time interaction as fixed effects, and fermenter as a random effect. Dry matter and NDF disappearance were higher in CAR fermenters compared to those receiving CON (69,7 % *vs* 66.0 %; $P<0.05$). Total volatile fatty acid (VFA) production was similar for both diets ($P>0.05$) as well as production and proportions of acetate, propionate, butyrate, isobutyrate, isovalerate and caproate; $P>0.05$). Consequently, Ac:Pr ratio was also similar ($P>0.05$) in both diets. Additionally, ammonia production and pH values were not different (152 vs 144 mg/d; 6.65 *vs* 6.70 for CON and CAR, respectively) and no differences were found in methane production or methane:VFA ratio. The results indicate that carrots can be included in a concentrate for dairy sheep replacing the whole amount of maize without compromising rumen fermentation. Using discarded carrots instead of maize in ruminant diets would reduce the use of potentially human-edible ingredients in animal feeding.

Funding

Funding from the Spanish State Research Agency (AEI) and the European Regional Development Fund (Project PID2021-124648OB-C22) is gratefully acknowledged. N. Merino acknowledges the support of the Junta de Castilla y León for her predoctoral contract.

Amino acid balancing for sustainable nitrogen efficiency in lactating cows fed lower dietary crude protein

D. Martinez Del Olmo, E. Rostami, E.E. Gültepe, B. Forier
Kemin Europa NV, Herentals, Belgium (eren.gultepe@kemin.com)

This study aimed to evaluate the effects of high-crude protein (CP) versus low-CP diets, balanced with rumen-protected methionine (Met, KESSENT™) and lysine (Lys, LysiGEM™, Kemin Animal Nutrition and Health, Belgium) or bypass protein, on milk production, composition, nitrogen (N) efficiency, and environmental impact in early lactating Holstein cows. A total of 60 lactating cows (15 primiparous, 14 second parity, and 31 ≥3rd parity) were used in a randomized block design, with a 3-week pre-period and a 6-week treatment period. The cows were allocated to three treatments, with 20 cows per treatment and 20 blocks, stratified by parity and pre-period fat and protein corrected milk (FPCM) yield, feed efficiency, milk fat, protein percentage across treatments. However, the MetLys treatment resulted in slightly reduced dry matter intake (DMI) and milk lactose percentage. Lowering dietary CP from 165 to 155 g/kg DM and balancing with either bypass protein or rumen-protected Met and Lys significantly reduced milk urea excretion, which suggests positive environmental impacts by decreasing nitrogen emissions. The lowest milk urea concentration was found in cows fed the low-CP diet supplemented with rumen-protected Met and Lys. While urinary N excretion was not reduced, fecal N output and creatinine concentrations were lower in cows supplemented with Met and Lys. Supplementing a low-CP diet (155 g/kg DM; target metabolizable protein coverage with rumen-protected Met and Lys improved N use efficiency compared to the high-CP control group (165 g/kg DM) throughout the study. In conclusion, feeding a low-CP diet balanced with rumen-protected Met and Lys improves N and feed efficiency and reduces milk urea excretion without negatively affecting milk production or composition, offering environmental and economic benefits.

Ruminal fermentation of a dairy sheep diet including leeks in a Rusitec system

I. Mateos[1,2], A. Díaz[1,2], C. Saro[1,2], M.J. Ranilla[1,2]
[1]Universidad de León. Dpto. Producción Animal, León, Spain. [2]Instituto de Ganadería de Montaña, Grulleros, Spain (mjrang@unileon.es)

In Spain, leek is a traditional crop in many regions. The production of leeks generates a large amount of waste (mainly the outer leaves and stems), which tends to be bulky, fibrous, and are often not used in human food due to their harder texture and lower commercial value. However, the use of these by-products in animal feed is a way to take advantage of them and contribute to the sustainability of the food production chain, as it helps to avoid waste and reutilize materials that would otherwise be discarded. On the other hand, leeks contain sulfur compounds that may influence ruminal fermentation, and some have been associated with a reduction in methane production. The aim of this study was to evaluate the effect of replacing maize by leeks in a conventional dairy sheep diet on rumen fermentation characteristics in Rusitec fermenters. Two 1:1 forage:concentrate ratio diets were formulated: a control diet and another with 16 % of leek totally replacing maize in the control diet. Diets were formulated to have similar crude protein (16.6 %) and neutral detergent fibre (27.4 %) contents. Two identical 13-day incubation runs were carried out independently using 4 Rusitec fermenters, and each of the two diets (20 g dry matter/day) was randomly assigned to two fermenters. Ruminal contents (solid and liquid) of four rumen-cannulated non-lactating Assaf sheep were used to inoculate the Rusitec fermenters. After 7-d of diet adaptation, diet disappearance and fermentation parameters were assessed in four consecutive days. The PROC MIXED of the SAS was used to analyse the data as a mixed model with repeated measures using, including diet, incubation run, time and diet x time interaction as fixed effects, and fermenter as random effect. Dry matter and NDF disappearance were higher ($P < 0.001$) in fermenters receiving the diet with leeks compared to the control (66.7 % *vs* 71.1 % for DM, and 31.3 % *vs* 39.9 % for NDF; respectively). Total VFA production was also higher ($P<0.05$) in the leek-containing diet (120 *vs* 130 mmol/d), as well as production and proportions of acetate and propionate. Production and proportion of butyrate was higher ($P<0.05$) in vessels fed the control diet. However, Ac:Pr ratio, minor VFA production and proportions, methane production and methane:VFA ratio were similar ($P>0.05$) in both diets. The leek diet showed a significant increase ($P<0.001$) of ammonia production (152 *vs* 211 mg/d), with pH values also higher ($P<0.05$; 6.65 vs 6.70). The results indicate that leeks can be included in a concentrate for dairy sheep replacing the whole amount of maize without compromising *in vitro* rumen fermentation. Using leek residues instead of maize in ruminant diets can contribute to greater utilization of local resources while reducing environmental impact.

Funding

Funding from the Spanish State Research Agency (AEI) and the European Regional Development Fund (Project PID2021-124648OB-C22) and assistance from N. Merino and V. Rodríguez is gratefully acknowledged.

Use of ensiled stoned olive pomace in the diet of fattening lambs: effect on dry matter intake and growing rate

F.G. Cella, A. Mantino, M. Finocchi, T. Malizzi, E. Senatore, A. Silvi, A. Serra, M. Mele
University of Pisa, Pisa, Italy (marcello.mele@unipi.it)

Use of byproducts in ruminant feeding is considered an effective strategy to reduce both feeding costs and environmental impact. According to the literature, increasing circularity plays a key role in enhancing the benefits and mitigating the negative impacts of livestock production, particularly regarding land use, soil health, water quantity and quality, biodiversity, and climate change. However, to include agri-industrial byproducts in animal feeding, certain constraints must be overcome, including seasonality and perishability. Ensiling byproducts is considered a feasible strategy to preserve the nutritional characteristics of high-moisture byproducts, such as stoned olive pomace. The aim of the present study was to compare a control diet that was high in starch and low in fiber with two diets that were rich in digestible fiber and low in starch. These two diets included ensiled stoned olive pomace and other agri-food byproducts as substitutes for cereals and soybean meal. Eighteen Appenninica growing lambs (19.0 ± 2.0 kg) in the fattening phase were randomly assigned to three groups of six lambs each. After a two-week adaptation period, each lamb was housed in an individual pen and fed one of the following diets for 30 days: a conventional fattening diet based on a mixture of corn, barley, soybean meal, and grass hay (control, C); a fattening diet based on ensiled stoned olive pomace, wheat bran, beet pulp, and distillers (SD); and a fattening diet based on ensiled stoned olive pomace, wheat bran, beet pulp, and faba beans (SF). At the end of the fattening phase, the experimental groups did not differ in final live weight, average daily gain, average daily dry matter intake, or the percentage of dry matter intake per kilogram of live weight. Lambs fed ensiled olive pomace tended to drink more water than those on the control diet ($P = 0.07$), and the amount of water intake per kilogram of dry matter intake was significantly higher ($P < 0.05$) for lambs fed with the two diets including ensiled stoned olive pomace, likely due to the higher polyphenol content. In conclusion, including ensiled stoned olive pomace alongside other agri-industrial byproducts rich in digestible fiber and protein resulted in growth performance similar to that of lambs fed a conventional, starch-rich diet based on cereal grains and soybean meal. The use of ensiled stoned pomace in the diet of growing lambs is thus a feasible strategy to reduce environmental impact of meat production without affecting animal performance.

Funding
This study was carried out within the Agritech National Research Center and received funding from the European Union Next-GenerationEU (PIANO NAZIONALE DI RIPRESA E RESILIENZA (PNRR) – MISSIONE 4 COMPONENTE 2, INVESTIMENTO 1.4 – D.D. 1032 17/06/2022, CN00000022).

Nutritional strategies to improve nitrogen retention in growing pigs under heat stress condition

M.O. Wellington[1], P. Bikker[2], A.J.M. Jansman[2]
[1]Trouw Nutrition R&D, Boxmeer, Netherlands. [2]Wageningen University & Research, Wageningen Livestock Research, Wageningen, Netherlands (michael.wellington@trouwnutrition.com)

Recently, it was shown that heat stress (HS) conditions significantly reduced nitrogen (N) retention in growing pigs. The HS-induced reduction in N-retention can potentially be explained by either a reduced availability of all amino acids (AA) or specific AA. The present study aimed to evaluate the impact HS on AA metabolism and N-retention and evaluate the nutritional strategy of a higher supply of all AA, glycogenic AA or a higher starch to spare glycogenic AA in alleviating the negative effect of HS on N-retention. A total of 80 barrows (21±0.7 kg BW) were used in a 28-day experiment, with 14-d *ad-libitum* feeding and growth performance followed by two N-balance periods of 5 days each at restricted feeding to account for HS effect on feed intake reduction. All pigs were housed under thermoneutral (TN; 24°C) conditions for 5 days in two rooms (TN vs. HS) before a gradual temperature increase in the HS room until the desired temperature (34°C). The diets were T1; starch 350 g/kg and crude protein (CP) 148 g/kg, T2; starch 500 g/kg and CP 148 g/kg, T3; starch 350 g/kg and CP 188 g/kg, T4; starch 500 g/kg and CP 188 g/kg and T5; starch 350 g/kg and CP 195 g/kg with extra glutamine, arginine and cysteine (n=8 pigs/treatment/room). Water intake was higher in the HS compared to TN pigs (6.8 vs. 2.8 L/d; $P<0.05$). On d7, bodyweight (BW) tended to be higher in T3, T4, and T5 ($P=0.06$), compared to T1 and T2 irrespective of the room temperature. On d14, BW was higher in TN compared to HS pigs ($P<0.05$; 32 vs 27.2 kg) with no diet effects. For growth performance (d0-14), average daily gain was higher in TN than HS pigs ($P<0.05$;783 vs 441 g/d) with a tendency for T3, T4, and T5 ($P=0.08$) to improve average daily gain under both TN and HS conditions. Similarly, average daily feed intake was higher in TN than HS pigs (1208 vs. 866 g/d; $P<0.05$) with no diet effects. For gain-to-feed (G:F), both temperature and diets had significant effects ($P<0.05$). The TN pigs had a higher G:F than HS pigs (0.64 vs. 0.49 g/g; $P<0.05$), while in both HS and TN rooms, T3, T4, and T5 had a higher G:F than T1 and T2 ($P<0.05$). In summary, HS reduced feed intake, daily gain, and G:F, whereas T3, T4, and T5 tended to recover the performance losses occasioned by the heat stress treatment. The results of the N-balance will be available before the conference, and then further conclusions can be made in line with the hypothesis.

Funding

This research was conducted within the public-private partnership “Adaptation of pig and poultry production to heat stress” (TKI-Agrifood LWV21.148), partially funded by the Ministry of Agriculture, Fisheries, Food Security and Nature (project BO-63-001-054), and by a consortium of private partners (Elanco Netherlands, Adisseo France, Eurolysine, Cobb Europe, and Nutreco Netherlands).

Effect of thermal heat stress on dietary energy utilization in growing calves

E. Labussiere[1], D. Bastien[2], M. Chanteperdrix[3]
[1]PEGASE, INRAE, Institut Agro, Saint-Gilles, France. [2]CIRVEAU, Idele, Mauron, France. [3]Idele, Le Rheu, France (etienne.labussiere@inrae.fr)

The occurrence of more frequent and more intense heat waves in the future will induce heat stress in farm animals. For those raised in closed buildings, such as veal calves, it is necessary to understand the adaptation strategies developed by animals to face heat stress. The objective of the experiment was to measure the effects of ambient temperature on behavior, feed intake, growth, nutrient balance and heat production and the effects of ambient temperature and relative humidity on heat loss in growing calves. After three weeks of adaptation to ambient temperature, 24 growing calves (mean body weight, BW = 136 kg, mean age = 109 +/- 7 days) were housed individually during 10 days in an open-circuit respiration chamber, where ambient temperature was set at 15, 20, 25 or 30°C (6 calves per condition). After one day, the relative humidity of the air varied every three days between 60, 70 and 80% according to a latin square design for each level of temperature. The calves were fed a liquid milk replacer twice daily and had free access to solid feed and water. Feed and water intake were recorded daily. Feces and urine were collected over the 10 days. The dynamics of heat production and heat loss as evaporated water were analyzed at every 10 second intervals. The data was analyzed for the effects of ambient temperature, relative humidity and their interactions. The intake of milk replacer did not significantly vary between ambient conditions and averaged 1309 g DM/d. Solid feed intake significantly decreased between 25 and 30°C from 1624 to 1346 g DM/d ($P = 0.03$). Water intake increased from 1.7 to 8.3 L/d when ambient temperature increased from 20 to 30°C ($P < 0.01$). The metabolizable energy intake significantly decreased when ambient temperature increased from 20 to 30°C ($P = 0.05$). Total heat production tended to decrease when ambient temperature was 30°C ($P = 0.10$) whereas time spent standing significantly increased ($P = 0.01$). The retained energy was not significantly affected by ambient temperature, but protein deposition significantly decreased when ambient temperature increased from 25 to 30°C ($P < 0.01$). The ambient temperature and the relative humidity inversely affected the proportion of heat lost as water evaporation, which increased from 41 to 79% between 15 and 30°C ($P < 0.01$), when relative humidity was 60%. The increase in relative humidity by 10% resulted in a 2.4% decrease in the proportion of heat lost as water evaporation ($P < 0.01$). These mean values will be further analyzed to take into account the dynamics of feeding and drinking behavior in relation to the instantaneous energy status and thermal balance of calves.

Funding

This work was realized within the scope of VOLCAN, a project funded by Carnot France Futur Elevage.

Both short- and long-term heat stress reduce post-absorptive nitrogen utilisation in growing pigs

A.E.P. Mary, A.J.M. Jansman, P. Bikker
Wageningen University & Research, Wageningen Livestock Research, Wageningen, Netherlands (paul.bikker@wur.nl)

Heat stress (HS) poses a significant threat to swine production, particularly as climate change increases the incidence of hot periods. This study aimed to investigate short- and long-term effects of HS on pigs and their capacity to adapt and recover, compared to thermoneutral (TN) conditions. After a 7-day adaptation period at 22°C, 40 male pigs (initial body weight 30.2±0.5 kg) housed in metabolic pens for 28 days were assigned to one of four experimental groups in a factorial arrangement with ambient temperature in Period 1, Day 8-18 (TN, 22°C and HS, 35°C) and Period 2, Day 19-26 (TN, 22°C and HS, 35°C) as respective factors. Ambient temperature for HS in Period 1 was gradually increased to 35°C over four days whereas TN and HS in Period 2 were realised by an abrupt change-over for half of the pigs of either treatment group in Period 1. The factorial arrangement allowed to assess the effects of short- (1 week) and long-term (2 weeks) HS, and recovery in TN conditions on body weight gain, nutrient digestibility, and nitrogen retention and utilization. All pigs were fed restrictively at 2.8 times their maintenance requirements for energy (293 MJ NE/kg BW), to allow the evaluation of direct effects of HS without interference of HS induced reduction in feed intake. Faeces and urine were quantitatively collected during two nitrogen balance (NB) periods: Day 12-17 (NB1) and Day 20-25 (NB2). Data were statistically analysed by ANOVA using a factorial arrangement with ambient temperature (TN) and (HS), in Period 1 and 2 as respective factors, litter of origin as random factor, and pig as experimental unit. A drastic increase in respiratory rate ($P<0.001$) and skin temperature ($P<0.001$) in HS pigs in both periods to increase heat dissipation confirmed the impact of HS on physiological responses and adaptation of the pigs. Overall body weight gain (771±24 g/d) and feed intake (1304±17 g/d) were similar across treatment groups. Water intake was increased by 2.2 and 2.5 L/d ($P<0.001$) by HS in Period 1 and 2, respectively. Nitrogen retention and utilization were reduced by HS in both balance periods. Post-absorptive nitrogen utilisation (N retention/digestible N intake) was reduced by HS from 79.6 to 73.5% and from 79.3 to 72.7% in NB1 and NB2, respectively. These results indicate that N-utilization in HS pigs was reduced, possibly due to reduced allocation of energy to protein deposition, increased protein turnover, or increased demand of amino acids for specific purposes, e.g. endogenous secretion or gluconeogenesis. This rewards study of targeted nutritional strategies to mitigate the impact of HS on N metabolism of pigs and support their adaptation capacity to high ambient temperatures.

Funding

This research was conducted within the public private partnership “Adaptation of pig and poultry production to heat stress” (TKI-Agrifood LWV21.148), funded by the Ministry of Agriculture, Fisheries, Food Security and Nature (project BO-63-001-054), and a consortium of five private partners (Elanco Netherlands, Adisseo France, Eurolysine, Cobb Europe, and Nutreco Netherlands).

Protein fractional synthesis rates under heat stress in growing crossbreed and Iberian pigs

L. Sarri[1], E. Labussière[2], H. Rajaei-Sharifabadi[1], J. Balcells[1], A.R. Seradj[1], G. de la Fuente[1]
[1]Universitat de Lleida – Agrotecnio-CERCA, Lleida, Spain. [2]3P and PEGASE, INRAE, Saint-Gilles, France (laura.sarri@udl.cat)

Reduced growth rates in pigs exposed to heat stress (HS) has traditionally been associated with significantly reduced feeding level to avoid additional heat production, with consequent potential nutrient imbalances. However, the metabolic alterations induced by the HS itself may be directly responsible of such imbalances. In terms of protein metabolism, HS-exposed pigs exhibit an upregulation of genes and biomarkers involved in protein catabolism, while genes involved in protein synthesis have been shown to be downregulated (Huau et al., 2024), explaining the reduced protein deposition. The use of stable isotope tracers to measure protein fractional synthesis rates (FSR) provides a more direct monitoring of protein metabolism *in vivo* that may enhance our understanding of these alterations. Furthermore, it has been suggested that intensive genetic selection to improve production performance may reduce the tolerance to HS, in contrast to other more rustic pig breeds adapted to their environment, such as the Iberian (IB). Therefore, a 2 × 2 factorial arrangement was designed with 11 crossbreeds (CO; Pietrain × (Landrace × Large White)) and 11 IB pigs, exposed to HS (5 IB and 5 CO; 33.5ºC) or thermoneutrality (6 IB and 6 CO; 23.5ºC) for 28 days. During the last 5 experimental days, nitrogen (N) balance was accurately measured, and on the last day a flooding dose of phenylalanine containing 15% $^{13}C_6$-Phe was administered intravenously, followed by blood sampling and sacrifice of the pigs. Samples of skeletal muscle, liver, and intestine were collected and analyzed by UPLC-tandem mass spectrometry, and the data were processed using the PROC MIXED procedure of SAS with environmental condition, breed, and their interaction as fixed effects. As expected, growth was significantly decelerated under HS, as was the calculated N retention (−19 g of N/d from digested N on average for the 2 breeds, $P<0.001$). Regarding protein FSR data, they were slightly reduced under HS, with no significant differences, with the jejunum being the main tissue that decreased its values (−22.7%/day), evidencing the particular sensitivity of the intestine to these circumstances. Considering the long-acclimatization period to HS, the small differences between the two environmental conditions may correspond to an adaptation process in which FSR may have partly recovered after a major disturbance at the start of the challenge. In addition, few differences were found between the two breeds, reinforcing the idea that IB pigs are also highly sensitive to heat stress due to the thick subcutaneous fat layer (Pardo et al., 2022). However, the duodenum revealed a significantly lower protein FSR in IB pigs than in CO pigs (−22.12%/day, $P<0.001$).

References

Huau, G., Liaubet, L., Gourdine, J.L., Riquet, J., Renaudeau, D., 2024. BMC Genomics 25, 99. https://doi.org/10.1186/s12864-024-09999-1
Pardo, Z., Seiquer, I., Lachica, M., Nieto, R., Lara, L., Fernández Fígares, I., 2022. Journal of Thermal Biology 106, 103230. https://doi.org/10.1016/j.jtherbio.2022.103230

Funding

This research was supported by the Spanish Ministry of Science and Innovation (grant PID2021-127949OB-100). The PIGWEB project has received funding from the EU Horizon 2020 research and innovation program (grant agreement No 101004770).

Effect of supplementation of Iberian lactating sows with betaine or amino acids on performance, metabolic and redox status under heat stress conditions

C. García-Contreras[1], A. Román[1], I. Fernández-Fígares[1], M. Lachica[1], F. Sánchez-Esquiliche[2], L. Ramírez[2], F. Gómez-Carballar[2], R. Nieto[1]
[1]Estación Experimental del Zaidín (CSIC), Granada, Spain. [2]Sánchez Romero Carvajal Jabugo, Huleva, Spain (consolacion.garcia@eez.csic.es)

Lactating sows are highly susceptible to heat stress due to the elevated metabolic heat generated during milk production. This can result in decreased feed intake, low milk yield, impaired reproductive performance and a slow piglet growth rate. The effects of supplementing lactating Iberian sows from day 70th of pregnancy onwards with betaine (BET; n=29) or amino acids (AA; n=27; arginine + valine) as nutritional strategies to mitigate heat stress was evaluated against a non-supplemented group (C; n=31) under heat stress (HS; n=47) and thermoneutral (NO-HS; n=40) conditions, in primiparous (P; n=35) and multiparous (M; n=52) sows. The average maximum temperature was 32.3ºC under HS and 23.7ºC for NO-HS. Productive parameters of lactating sows were analysed, including sow weight at farrowing and weaning, weight loss during lactation, daily feed intake, loin depth and back-fat thickness at weaning, litter size, average litter weight (at farrowing and weaning), and average daily gain of piglets (n=409). blood samples were collected at weaning, from a representative group of sows (n=58) for analysis of plasma biochemical parameters and redox status (FRAP and TBARs). Both at farrowing and weaning, M sows were heavier than P sows, and NO-HS sows were heavier than HS sows (P<0.001). A reduction in daily feed intake was observed under HS (by 6%; P<0.001). However, no significant differences were found in sow weight loss during lactation or litter size. HS decreased average litter weight at birth and at weaning (P<0.005). Moreover, piglets under HS showed lower ADG (P<0.001), although a treatment × parity interaction was observed, so that piglets from M sows supplemented with betaine showed greater lactation ADG compared with piglets from C multiparous sows while piglets from AA multiparous sows were in an intermediate position (P<0.05). Sows in the BET group showed lower back-fat thickness compared with the C group (P<0.05) and higher plasma concentration of total cholesterol and HDL-cholesterol (P<0.05). These results suggest greater body fat mobilization in the BET group sows. A trend towards greater loin depth was also observed in the BET group (P=0.077), along with a treatment × HS interaction, with greater loin depth in BET sows under HS (P<0.001) compared with AA or C sows. Under NO-HS, plasma glucose concentration was higher in the BET group than in the C group (P<0.01). Finally, maternal plasma redox status parameters were not affected by the treatment. Nevertheless, lower antioxidant capacity and higher oxidative stress parameters were observed in plasma during HS (P<0.001). HS caused a decreased in lactation performance in Iberian sows that could be reduced by dietary betaine addition.

Funding

Funded by Spanish Ministry of Science and Innovation (PID2021-125059OB-I00 and JDC2022-049444-I).

In vitro methane production kinetics of red clover and grass silages used in dairy cow diets

O. Pitkänen[1], A. Halmemies-Beauchet-Filleau[1], S. E. Räisänen[1,2], Þ.H. Sigurðardóttir[1], T. Kokkonen[1], A. Vanhatalo[1]

[1]University of Helsinki, Department of Agricultural Sciences, Helsinki, Finland. [2]2Department of Animal and Veterinary Sciences, Research Centre Foulum, Aarhus University, Tjele, Denmark (aila.vanhatalo@helsinki.fi)

Enhancing the proportion of red clover (*Trifolium pratence*) in grass swards offers multiple agronomic benefits like atmospheric nitrogen binding. We investigated fermentation kinetics and CH_4 production of red clover silage in comparison to multispecies (50% *Phleum pratense*, 15% × *Festulolium*, 15% *Lolium perenne,* 10% *Festuca arundinacea*, 10% *Festuca pratensis*) grass silage using an automatic Gas Endeavour *in vitro* batch system. First cut wilted and formic acid-preserved silages were freeze-dried before incubation. The six experimental diets were as follows: pure grass silage (PG), pure red clover silage (PR), grass silage, barley, and oats (GC), red clover and grass silages, barley and oats (RGC), GC diet with faba bean (GCF), and RGC diet with faba bean (RGCF). In the diets with multiple feeds, red clover and grass silages were mixed 2:1, concentrates and silages 2:3, oat and barley 1:1, and oat, barley, and faba bean 3:3:4. Feeds were incubated for 22 h in an incubation medium of dairy cow rumen fluid and McDougall's buffer solution (1:2). Total gas and CH_4 productions were continuously measured. The experiment was repeated in 4 replicates and data analysed using SAS Mixed procedure. Both silages were of good quality, but red clover silage was less extensively fermented and had more crude protein (18.9% vs. 14.6%) and less NDF (34.0 vs. 51.2%) than grass silage. After incubation, diets including concentrates had lower pH and less residual dry matter than pure silage diets. Red clover and faba bean increased final pH of incubation media but did not affect dry matter disappearance. Total gas production was greater for PR than for PG, whereas it was smaller for RGC and RGCF than for GC and GCF. Diets with concentrates produced more gas than pure silages. Faba bean increased gas production only when red clover was not included (i.e. GCF vs. GC). Red clover did not affect CH_4 production but inclusion of concentrates and faba bean increased CH_4 production by 28% and 48%, respectively, compared to pure grass diets. Red clover fermented faster than grass silage. Cumulative total gas production was 62% greater for PR than for PG at 4 h but only 11% greater at 22 h. Similarly, red clover increased the rate of CH_4 production by 110% although it did not increase CH_4 total production. Overall, the differences in gas production were smaller between the mixed diets (RGC and RGCF vs. GC and GCF) than between the pure silages (PR vs. PG). Faster fermentation of red clover silage in comparison to grass silage was consistent with existing literature (*e.g.* Kuoppala et al., 2009). Our results suggest that despite differences in rate of CH_4 production, the differences in total CH_4 production between the red clover and grass silage diets seem minor.

Reference

Kuoppala, K., Ahvenjärvi, S., Rinne, M., Vanhatalo, A., 2009. Journal of Dairy Science 92, 5634–5644. https://doi.org/10.3168/jds.2009-2250

Funding

The experiment was a part of Leg4Life project funded by the Strategic Research Council (SRC) established within the Academy of Finland.

Reducing the carbon footprint of broiler chickens through the choice of feed ingredients

J. Gickel[1], C. Ullrich[2], C. Visscher[2]
[1]Science and Innovation for Sustainable Poultry Production (WING), University of Veterinary Medicine Hannover, Foundation, Hannover, Germany. [2]Institute of Animal Nutrition, University of Veterinary Medicine Hannover, Foundation, Hannover, Germany (julia.gickel@tiho-hannover.de)

The carbon footprint of broiler chickens is largely influenced by the feeding of the animals. Therefore, efforts are being made to reduce the carbon footprint of the diets. In this study, four scenarios (A to D) were set up based on a 3-phase control diet used in a feeding trial beforehand. Starter and grower phase lasted 7 days each, followed by 21 days finisher phase. The control diet was based on wheat (Germany), soybean meal (South America), corn (Germany) and sunflower oil (Germany). Minerals as well as amino acids (arginine/lysine/methionine/threonine) and phytase were added to fulfill the requirements of the broiler chickens. All diets in all scenarios contained the same amount of metabolizable energy (12.5/13.0/13.1 MJ per kg) and crude protein (215/202/196 g per kg) and comparable amounts of phosphorus, methionine, threonine and a constant arginine-lysine ratio (all based on 88% dry matter). In scenario A the same ingredients as in the control diets were used but the feed was optimized for a low carbon footprint instead of price. In scenario B the protein source was changed by adding peas, broad beans, lupins and rapeseed meal (all Germany) to the diets and scenario C the energy source was changed by adding former food dry products, wheat bran, sugar beet molasses, yeast (all Germany) and animal fat from pig (Europe) to the diets. Scenario D allowed all ingredients from scenario B and C for the optimization. All diet were optimized for a low carbon footprint (per kg feed) using the values from the Global Feed LCA Institute (GFLI) database (2024). In addition, the carbon footprint per kg carcass weight (according to ISO 14040) was assessed using the online software application Opteinics™ operating based on the GFLI database. Therefore, it was assumed that all scenarios resulted in the same performance as the control scenario. The carbon footprint of the single diets (per kg feed) used in the scenarios resulted to be 3.6-6.4% (A), 39.0-44.8% (B), 1.1-9.9% (C) and 57.0-58.5% (D) lower than the carbon footprint of the diets in the control scenario. The carbon of the broiler chickens in the control scenario resulted to be 2.86 kg CO_2 eq per kg carcass weight and was reduced to 2.79 (A), 2.65 (B), 2.51 (C) and 2.35 (D) kg CO_2 eq per kg carcass weight in the scenarios. It was shown that the carbon footprint of broiler chickens could be reduced through the choice of ingredients by up to 17.8%.

Dynamic shifts in water requirements of breeding bulls across physiological states of body weight loss and compensatory growth

M. A. Fonseca[1,2], N.F. De Paula[3,1,2], L.R. Bezerra[4,1,2], D.C. Abreu[5,1,2]
[1]Clayton Livestock Research Center, Clayton, USA. [2]New Mexico State University, Las Cruces, USA. [3]Federal University of Mato Grosso, Cuiabá, Brazil. [4]Federal University of Campina Grande, Patos, Brazil. [5]Federal University of Mato Grosso, Sinop, Brazil (mozart@nmsu.edu)

Water is a critical but often overlooked nutrient in livestock systems, particularly for breeding bulls experiencing dynamic physiological states such as body weight (BW) loss and compensatory growth. This study evaluated the interrelationships between water intake (WI; L/day and L/kg$^{0.75}$) and energy requirements during distinct physiological phases in breeding bulls to better understand how fluctuations in net energy for maintenance (NEm), gain (NEg), and total energy (NEt) influence WI. Thirteen crossbred Angus × Hereford bulls (29.6 ± 3.4 months.; initial BW = 715 ± 36.1 kg) were housed in a controlled feedlot environment equipped with automated waterers over a 198-day period. The feeding strategy was designed to impose four phases: BW maintenance (Phase 1), BW loss (Phase 2), post-BW loss maintenance (Phase 3), and compensatory growth (Phase 4). Bulls were fed beardless wheat hay and a mineral mix, while daily measurements of individual WI, BW, and environmental variables were recorded. Multivariate analyses and principal component analyses (PCA) were used to evaluate the complex relationships among performance metrics, WI, and energy demands. During BW loss (Phase 2), bulls exhibited significant reductions in WI, metabolic BW (MBW), average daily gain (ADG), dry matter intake (DMI), DMI/kg$^{0.75}$, WI/kg$^{0.75}$, NEm, NEg, NEt, body condition score (BCS), back-fat thickness (BFT), and ribeye area (RA) relative to initial maintenance (Phase 1). Conversely, compensatory growth (Phase 4) showed substantial increases in all aforementioned variables compared to post-restriction maintenance (Phase 3). These findings underscore the plasticity of WI in response to physiological stress and refeeding, with WI closely tracking energy flux and tissue accretion patterns. The PCA revealed that WI, energy variables, and environmental conditions do not operate independently but rather exhibit integrated patterns that vary by physiological state. Thus, oversimplified models of WI may fail to capture the nuance needed to accurately predict water use across production systems. The results indicate that energy requirements during BW loss and gain critically influence WI, reinforcing the need for context-specific estimates that account for environmental conditions, energy metabolism, and body composition dynamics. Understanding these interdependent relationships can improve the precision and accuracy of water requirement models and inform strategies to optimize water use efficiency in beef production systems, ultimately contributing to more sustainable resource management.

Funding

The authors would like to thank the USDA-NIFA Grant # 2018-67016-27912 for supporting this study.

A dose-response study on different dietary energy levels in combination with two protein levels on broiler performance

K. Buyse[1], J. Buyse[2], E. Delezie[1]
[1]ILVO, Merelbeke-Melle, Belgium. [2]KULeuven, Leuven, Belgium (Kobe.Buyse@ilvo.vlaanderen.be)

Optimizing feed energy is crucial as rising feed costs burdens the industry. Current energy calculations need revision due to improved broiler genetics, housing, and management. Balancing energy with protein reduces nitrogen excretion. This study examined the impact of energy and protein density on broiler performance. In total 1,680 male broilers (Ross308) were allocated to 70 pens. During the starter phase (d1-10), birds received a standard diet. From the grower phase, 7 energy-levels were formulated in combination with 2 protein levels (14 doses; n=5). Metabolizable energy levels ranged from 10.46 to 12.67 MJ/kg in combination with 19.5% (ConCP) or 17.5% crude protein (LowCP) in the pelleted grower (d10-26); and from 10.54 to 12.77 MJ/kg in combination with 19% (ConCP) or 17% crude (LowCP) protein in the pelleted finisher (d27-43) diets. Pen bodyweight (g), average daily gain (g/d), average daily feed intake (g/d) and feed conversion (FCR) were calculated per phase, corrected for mortality. Litter ammonia levels (%) were measured at day 37. The data was assessed with linear regression with energy (MJ/kg feed) as continuous independent variable interacting with CP as discrete independent variable. Growth rate and bodyweight in the grower and finisher showed a significant interaction between energy density and protein levels with a slope for LowCP and ConCP of -44.71 g/MJ.kg (gram per energy content) and -80.39 g/MJ.kg (p=0.011), respectively for grower and -129.68 g/MJ.kg and 216.72 g/MJ.kg (p=0.012), respectively in finisher diets. Within the tested energy range, bodyweight was significantly higher in de ConCP groups (<0.001; both grower and finisher). Feed intake was independent of protein leveland showed an overall negative slope of -4.18 g/MJ.kg (LowCP) and -5.50 g/MJ.kg (ConCP) in the grower (p=0.219) and -11.51 g/MJ.kg (LowCP) and -13.64 g/MJ.kg (ConCP) in the finisher (p=0.358). Feed intake was higher in ConCP (p<0.001; both grower and finisher). These results suggested a compensatory feed intake with decreasing dietary energy. This was reflected in FCR with a small but significant dependency on CP with -0.01 units/MJ.kg (LowCP) and 0.01 units/MJ.kg (ConCP) in the grower (p=0.038) and no significant interaction in the finisher (p=0.426): -0.03 units/MJ.kg (LowCP) versus-0.01 units/MJ.kg (ConCP). Mean FCR-values were higher for the lowCP (p<0.001; both grower and finisher). Litter ammonia levels (%) showed a decreasing slope with increasing energy levels (-0.7%/MJ.kg; p<0.001) with no interaction of CP. There was a 1.04% decrease from ConCP to LowCP (p<0.001). In this study broilers compensated for energy density by higher feed intake. This affected the ingested energy:protein ratio and therefore resulted in a decreasing growth rate, bodyweight and litter ammonia with increasing dietary energy density. This effect was more pronounced with higher dietary CP-levels.

Low dietary protein and low or zero soybean meal diets in broilers: performance and sustainability implications

B. Saremi, B. Matton
CJ Europe, Frankfurt am Main, Germany (b.saremi@cj.net)

In Europe, sustainability is a major concern for animal industry. Soybean meal (SBM) is a major imported protein source in EU with a negative impact on sustainability specially if it is sourced from locations with land use change history. There are two ways to reduce SBM in feed i.e. lowering crude protein (CP) in feed or replacing SBM with other local protein sources in feed. In a three phase broiler study (0-12, 13-21, 22-35 days), 1350 Ross 308 birds were divided between 15 pen replicates per treatment. The dietary CP was reduced either only in the finisher phase (19% to 17%, T2), in both the grower phase (20% to 19%, T3) and finisher phase, or in three phases (21% to 20%, starter phase, T4). In two additional groups, SBM inclusion was reduced either to a maximum of 15% SBM (low, T5) or 0% SBM (T6) compared to a positive control (T1) group (Aviagen 2019). The body weight (BW), daily weight gain (DWG), daily feed intake (DFI) and feed conversion ratio (FCR) were measured in each phase. At day 36, 4 birds per pen were slaughtered for carcass evaluation. Foot pad lesion scoring (FPS) was done at day 22 and 35. Differences were studied with a linear regression model. Post-hoc pairwise comparisons were made without adjustment method ($P<0.05$). Global warming potential (GWP) was calculated based on Opteinics per treatment for different growth phases. The performance data are reported previously (Saremi et al. 2023). In summary, the low CP diets had no negative impact on performance parameters. Low and Zero SBM groups had a better performance compared with PC. In the starter phase, 1726, 1548, 1283, and 748 kg CO2 eq per ton of feed was produced in T1-3, T4, T5 and T6, respectively. In the grower phase, 1708, 1541, 1342, and 781 kg CO2 eq per ton of feed was produced in T1-2, T3-4, T5 and T6, respectively. In the finisher phase, 1612, 1262, 1335, and 810 kg CO2 eq per ton of feed was produced in T1, T2-4, T5 and T6, respectively. In conclusion, low CP diets are possible to be applied to broiler diets without any negative impacts on performance parameters. Low and zero SBM diets showed performance and sustainability superiority compared to a SBM based diet.

Reference

Saremi, B., Vlerick, L., Matton, B., Millecam, J., 2023. Proceeding of European Symposium of Poultry Nutrition, Rimini, Italy.

In vitro evaluation of bell pepper as a concentrate ingredient for dairy sheep diets

C. Saro[1,2], A. Díaz[1,2], I. Mateos[1,2], M.J. Ranilla[1,2]
[1]Universidad de León. Dpto. Producción Animal, León, Spain. [2]Instituto de Ganadería de Montaña, Grulleros, Spain (mjrang@unileon.es)

Bell pepper and chillies production reaches over 38 million t in the word. In Spain, bell pepper is the second most produced vegetable, only after tomatoes, leading the production in the EU. On average, about 46 % of the bell pepper fruits are suitable for first grade fresh market, 29 % go for processing and the remainder (25 %) are waste, being the second vegetable in order of the amount of waste produced after carrots. The use of bell pepper by-products and waste in ruminant feeding can be highly beneficial, both from an economic and environmental perspective. However, it is always important to carefully manage their inclusion in the animal diet to ensure that it does not negatively affect ruminal fermentation, health or the quality of the products. The aim of this study was to evaluate the effect of replacing maize by bell pepper in a conventional dairy sheep diet on rumen fermentation in Rusitec fermenters. Two 1:1 forage:concentrate ratio diets were formulated: a control diet and another with 16 % of bell pepper totally replacing maize in the control diet. Diets were formulated to have similar crude protein (16.6 %) and NDF (27.4 %) contents. Two identical 13-day incubation runs were carried out independently using 4 Rusitec fermenters, and each of the two diets (20 g dry matter/day) was randomly assigned to two fermenters. Ruminal contents of four rumen-cannulated non-lactating Assaf sheep were used to inoculate the Rusitec fermenters. After 7-d of diet adaptation, diet disappearance and fermentation parameters were assessed in four consecutive days. Data were analysed as a mixed model with repeated measures using the SAS software; diet, incubation run, time and diet x time interaction as fixed effects, and fermenter as a random effect. Dry matter and NDF disappearance were higher ($P<0.01$) in the vessels receiving pepper bells compared to those receiving the control diet (66.0 % *vs* 70.2 %; 31.3 % *vs* 38.7 %; respectively). Total VFA, acetate, propionate and valerate production were also higher ($P<0.05$) in the bell pepper diet, whereas VFA proportions were similar for most of them. Bell pepper diet showed a significant increase ($P<0.001$) of ammonia production. pH values were similar in both diets (6.65 *vs* 6.68). Methane production was higher ($P<0.05$) in the vessels that received the by-product (34.3 *vs* 36.5 mmol/d for control and pepper bell, respectively) but no differences were found methane:VFA ratio. The results indicate that bell pepper could replace maize in a concentrate for dairy sheep without negatively modifying *in vitro* rumen fermentation. If further confirmed, pepper by-products, when used properly, can be an excellent source of fibre and nutrients for ruminants, helping to improve the sustainability of livestock farming while optimizing available agricultural resources.

Funding

Funding from the Spanish State Research Agency (AEI) and the European Regional Development Fund (Project PID2021-124648OB-C22) and assistance from N. Merino and V. Rodríguez is gratefully acknowledged.

Effect of a silage inoculant on fermentation and protein quality in alfalfa silage

J. Witt[1], E. Kramer[1], E.S. Aniceto[2], S. K. Cronin[2], T.F. Gressley[2]
[1]ISF GmbH, Pinneberg, Germany. [2]Department of Animal and Food Science, University of Delaware, Newark, USA (johanna.witt@is-forschung.de)

Legumes such as alfalfa can play a special role in sustainable farming in the future due to their ability to bind nitrogen from the air. In addition, they are highly digestible, protein-rich feed for ruminants, but difficult to ensile due to their buffering capacity (Borreani et. al., 2018). According to Muck et al. (2018) the use of a silage inoculant with combined heterolactic and homolactic lactic acid bacteria helps to speed fermentation and reduce growth of undesirable bacteria and yeasts enhancing overall silage quality and aerobic stability. Therefore, this study aimed to evaluate the effects of a microbial inoculant on the fermentation profiles and protein quality of alfalfa in laboratory bag silos stored at 30°C. Alfalfa was harvested at late bud stage and wilted to 39% DM with 5.6% water-soluble carbohydrate and 19.8% crude protein. Two treatments in five replicates were performed: 1) CON – untreated control; 2) INO - 300,000 cfu/g of a mixture of *L. buchneri, L. plantarum* and *L. lactis* (Bonsilage Alfa). Bags were opened on day (d) 1, 2, 5, 7, 56, and 112 of ensiling and analyzed for nutrients, pH, enterobacteria, yeasts, silage acids and NH_3-N. Data were analyzed using Tukey 's Honestly Significant Difference or Student's t-test ($P \leq 0.05$). The pH was significantly lower for INO than CON at all time points with the largest difference at d 2 (CON: 6.34; INO: 4.65). Yeasts were significantly reduced for INO compared to CON at d 5 and d 7as well as enterobacteria (log cfu/g fresh weight) were significantly reduced for INO at d 2 (CON: 8.0; INO: 6.7), d 5 (CON: 3.0; INO: <2.0) and d 7 (CON: 2.7; INO: <2.0). Further NH_3-N (% DM) was significantly reduced for INO compared to CON at d 2, 7 and 56 (at d 56 CON: 0.16; INO: 0.09). Lactic acid (% DM) increased over time faster for INO than CON, and was significantly greater for INO at d 1, 2, 5, and 7 (at d 7 CON: 6.6; INO: 9.7). Due to the rapid increase in lactic acid and the related faster acidification for the treated silage compared to the control, the silage nutrient quality was better preserved. This is confirmed by the early reduction in enterobacteria and the significantly lower NH_3-N, indicating reduced protein degradation in the treated silage. The use of a silage inoculant is therefore ecologically beneficial as high-quality forage is produced in a resource-saving manner.

References

Borreani, G., Tabacco, E., Schmidt, R.J., Holmes, B.J., Muck, R.A., 2018. Journal of Dairy Science 101, 3952–3979. https://doi.org/10.3168/jds.2017-13837
Muck, R.E., Nadeau, E.M.G., McAllister, T.A., Contreras Govea, F.E., Santos, M.C., Kung Jr, L., 2018. Journal of Dairy Science 101, 3980–4000. https://doi.org/10.3168/jds.2017-13839

Funding

Development (CNPq, grants number 308779/2023-1, 408287/2021-7 and 442675/2023-2), and Evonik Operations GmbH, and Seara Alimentos - JBS Foods Brazil.

Ontogenesis of protein-energy metabolism in two divergent broiler lines selected for ultimate pH of the Pectoralis major muscle

A. Petit[1], N. Couroussé[1], E. Cailleau-Audouin[1], T. Bordeau[1], J. Delaveau[2], C. Rat[2], S. Tesseraud[1], S. Métayer-Coustard[1]
[1]INRAE, Université de Tours, BOA, Nouzilly, France. [2]INRAE, PEAT, Nouzilly, France (sonia.metayer-coustard@inrae.fr)

Early phenotypic and metabolic characterization of chickens, issued from a commercial fast-growing line and selected for ultimate pH (a proxy of muscle glycogen reserves) of the *Pectoralis major,* revealed that, although they did not differ at hatching in terms of weight and breast muscle yield, they already showed metabolic differences, probably related to variations in the nutrient availability and/or utilization *in ovo* (Métayer-Coustard et al., 2021). Fine characterization of the yolk and amniotic fluid by NMR showed quantitative and qualitative differences in the metabolites and nutrients potentially available to developing embryos, which may contribute to the metabolic and developmental differences observed after hatching between the pHu+ and pHu- lines (Petit et al., 2022). To determine the consequences of these different nutrient supplies on embryo metabolism, and to define the window during which metabolic orientation takes place in these two lines, the ontogeny of protein-energy metabolism was studied in the liver (a metabolic crossroads) at different stages of embryonic development (E12, E14, E18) and post-hatching (D0, D8). Eight clusters of genes showed a common expression profile between embryonic day 12 (E12) and post-hatch day 8 (D8). These clusters are not representative of any specific metabolic pathway or function. At E12 and E14, the majority of genes differentially expressed between the two lines were overexpressed in the pHu+ line. Our results highlighted a metabolic orientation for pHu+ line toward proteolysis, glycogen degradation, ATP synthesis, autophagy, probably in response to higher energy requirement than those of pHu- line. Conversely, the majority of genes differentially expressed from E18 were overexpressed in the pHu- line. During the metabolic switch at E18, there was a decrease in the expression of genes linked to several metabolic functions (e.g. protein synthesis, autophagy and mitochondrial activity). A gender effect was also observed for several metabolic pathways at D0 and D8. Metabolism appeared to be more active and oriented towards protein synthesis and fatty acid β-oxidation in males, while genes overexpressed in females are linked to carbohydrate metabolism. Our results highlighted that the metabolic orientations specific to the pHu+ and pHu- lines are established very early, probably in relation to their different genetic backgrounds and available nutrients. An indirect approach also revealed a distinct metabolic signature between pHu+ and pHu- embryos as early as E10 by NMR analysis of the chorioallantoic fluid in embryonated eggs. This fluid is considered to be a compartment that stores both the embryo's metabolic waste products of the embryos, free amino acids and other important compounds that can be reused for embryo nutrition at the end of development. The innovative aspect of this approach lies in the demonstration that the egg could serve as a source of relevant, early indicators or biomarkers of the animal's energy status or chick's robustness.

References

Métayer-Coustard, N., Lalmanach, S., Lahaye, L., Tesseraud, S., Tesseraud, L., Simon-Lecomte, E., 2021. Frontiers in Physiology 2, 643580. https://doi.org/10.3389/fphys.2021.643580
Petit, S., Rougière, N., Peralta, M.A.R., Médale, F., Métayer-Coustard, N., 2022. Scientific Reports 12, 5533. https://doi.org/10.1038/s41598-022-09509-x

Funding

This research and the PhD student were supported by INRAe and University of Tours.

Increasing the energy and lysine content of sow transition diets can improve litter performance and modulate colostrum composition and miRNA expression

D. Luise, A. Zurru, L. Laghi, F. Correa, P. Trevisi
Department of Agricultural and Food Sciences, University of Bologna, Bologna, Italy (diana.luise2@unibo.it)

The transition period from 7 days before parturition to 4 days after parturition is a critical period for the sow due to physiological changes and nutritional requirements. A balanced diet in terms of energy and amino acids (AAs) could improve reproductive performance, colostrum quality and piglet growth. The aim of this study was to evaluate the efficacy of a sows transition diet with higher energy and AAs quantity, compared with a standard diet, on the composition and quality of colostrum and on sow and piglet performance. A total of 100 sows, balanced for body condition score (BCS), muscle and backfat depth and parity, were divided into the CO and TRT groups 7 days prepartum. The CO diet had 2950 kcal metabolized energy (ME) and 0.70% SID lysine (Lys) and a TRT diet had 3100 kcal ME and 0.85% SID Lys. The AAs/SID Lys ration was maintained in both diets. Diets were administered from day 6 prepartum to day 4 postpartum. The duration of farrowing, the interval between births and the vitality of piglets were recorded for 12 sows/group. Performance and colostrum data (20 sows/group) were collected at farrowing (d0), day 6 (d6) and weaning (d24). Colostrum was analysed for proximal, immunoglobulins, somatic cell count (SCC), metabolomic composition (NMR analysis) and microRNA (miRNA; 10 sows/group) abundance. The diet did not affect feed intake, BCS, backfat and muscle loss of the sows. Farrowing duration and piglet birth intervals did not differ. The TRT group tended to have a higher number of live births ($P=0.066$), a lower number of stillbirths ($P=0.002$) an increase in piglet weight at weaning ($P=0.032$) and average daily gain d0-d24 ($P=0.080$) compared with the CO group. Piglet mortality during the suckling period did not differ. Colostrum from the TRT group tended to have a higher SCC ($P=0.07$) and had a higher fat% ($P=0.036$) and a different metabolomic profile, mainly characterised by a higher concentration of UDP-gluconate and carnitine and a lower concentration of citrate ($P<0.05$), highlighting the effect of diet on the energetic metabolism of the sow. A total of 208 miRNA were detected in colostrum and 13 were differentially expressed between the 2 groups ($P<0.05$). The TRT group had a higher ssc-miR-143-3p expression, which is associated with increased phagocytosis by myeloid and granulocytic cells, which may explain the increased SCC. In addition, ssc-miR-143-3p was associated with reduced inflammation and oxidative stress. This, together with the increase in fat and specific metabolites, could potentially benefit piglet performance. In conclusion, the results suggest that a transition diet consisting of 3,101.08 Kcal EM and 0.85% SID Lys is capable of improving sow parturition performance (fewer stillbirths) and lactation performance by modifying sow energy metabolism and colostrum quality.

Funding

Research supported by SustainableHeavySuis Project (J33C23002980002) - L.R. n. 17/2022-DGR 165 del 06/02/2023, Emilia Romagna Region, Italy. The authors thank Gruppo Veronesi Srl for the support in managing the *in vivo* trial.

Metabolic pathways involved in the effects of guanidinoacetic acid supplementation to sows on parturition and piglet development

P. Loibl[1], B.B.D. Muro[2,3], R.F. Carnevale[2,3], R. Yao[3,4], M. Malik[3], C.A.P. Garbossa[2], G.P.J. Janssens[4], D. Maes[3]
[1]Alzchem Trostberg GmbH, Trostberg, Germany. [2]Department of Nutrition and Animal Production, School of Veterinary Medicine and Animal Sciences, University of São Paulo, São Paulo, Brazil. [3]Department of Internal Medicine, Reproduction and Population Medicine, Faculty of Veterinary Medicine, Ghent University, Ghent, Belgium. [4]Department of Veterinary and Biosciences, Faculty of Veterinary Medicine, Ghent University, Ghent, Belgium (dominiek.maes@ugent.be)

The peripartal period stresses the sow's metabolism critically as energy requirements increase due to preparation for and onset of farrowing (1). Guanidinoacetic acid (GAA) acts as a source of creatine to support the energy metabolism of sows. GAA, however, needs to be methylated to creatine, thereby being a potential critical factor in the peripartal period as it presents peak methylation activities in sows (2). This study aimed to examine the effects of supplemental GAA in the peripartal and lactation period on metabolic pathways in sows and their piglets. The experiment was run on a practical piglet production farm in Belgium. In total, 23 hyperprolific sows (Landrace × Yorkshire, Danbred®) were assigned to two different dietary treatments (Control or Control + 0.1% GAA (Creamino®, Alzchem Trostberg GmbH) "on top"). Experimental feeds were fed from the transfer to the maternity unit 5 days pre-farrowing until weaning. Next to measurements of sows' and piglets' performance (body weight development of sows and piglets, farrowing kinetics, litter size), piglets' and sows' blood and/or colostrum and milk samples were taken at different time points (one day prepartum, one day postpartum, the 14th day of lactation, and the 28th day of lactation) during the experiment for targeted metabolite analysis. Non-dystotic sows farrowed significantly quicker when receiving GAA (419 min, Control, 286 min, GAA). Piglets born to GAA-supplemented sows exhibited greater weight gain (+40 g) from birth to 24 hours after birth ($p < 0.05$) but were significantly lighter at weaning (-290 g) compared to piglets from sows receiving the control diet. However, the piglets from GAA sows exhibited a lower mortality rate (35.0%, control, 22.1%, GAA). Dietary GAA altered the profiles of plasma amino acids and acylcarnitines in piglets as well as sows. These metabolic effects varied on the different timepoints. GAA's effects exceeded its role as the precursor of creatine. For instance, it enhanced methylation related pathways and the amino acid metabolism only five days after the onset of supplementation. This was obvious due to an increased methionine to homocysteine ratio indicating higher methylation capacity. In later stages the significant effects of supplemental GAA indicated a reduction in muscle and fat catabolism in the respective sows, whereas it promoted fat catabolism in late lactation. The alterations in these metabolic profiles suggest that GAA supplementation may enhance the overall metabolic efficiency of sows and piglets, potentially leading to improved health and performance. In conclusion, GAA supplementation could positively influence sows' productivity and support the energetic status of sows in specific production stages.

References

Feyera, T., Theil, P.K., 2017. Livestock Science 201, 50-57. https://doi.org/10.1016/j.livsci.2017.05.001
van Riet, M.M.J., Millet, S., Langendries, K.C.M., van Zelst, B.D., Janssens, G.P.J., 2019. Journal of Animal Physiology and Animal Nutrition 103, 858-867. https://doi.org/10.1111/jpn.13078

Quantifying variation in nutrigenomics: evidence from pig studies

E.F. Knol[1], M. Siwek[2]
[1]Topigs Norsvin Research Center, Den Bosch, Netherlands. [2]Bydgoszcz University of Science and Technology. Department of Animal Biotechnology and Genetics., Bydgoszcz, Poland (egbert.knol@topigsnorsvin.com)

Phenotype equals Genotype + Environment, nutrition and genetics decide greatly the performance of livestock. Nutrigenomics focuses on how diet affects gene expression. Nutrigenomics is also about epigenetics as one of the paths of non-mendelian inheritance. In layman terms: it can be considered as a last inherited adaptation to the environment the offspring can expect. Nutrition. The direct effects of nutrition are self-evident. Interesting are early life experiences, nutritional (or other) factors that change individuals in early development (e.g. in utero) for the rest of their life, e.g. colostrum quantity can influence myofiber maturation. Even more interesting are transgenerational effects, influences of nutrition that last across generations. Genetics. Animal breeders estimate the influence of genetics on the phenotype in environments which are as constant as possible and try to correct for the remaining variation by adding Herd Year Season (HYS) effects to their models. Gradually these models have become more complex, e.g. by adding genotype x environment interactions, adding maternal and social interaction effects to the equations. Nutrigenetic studies evaluate how genes and dietary components interact. Goal of this paper is to narrow the gap between nutrition and genetics. The obvious aim to study epigenetic effects is to understand how environmental factors can influence gene expression without altering the DNA sequence itself. Understanding the underlying principles means focusing on mechanisms such as DNA methylation, histone modification, and non-coding (micro) RNA. With modern tools such as whole genome bisulphite sequencing, chromatin immunoprecipitation, and RNA sequencing, variation in these mechanisms can be assessed, the results can be seen as intermediate phenotypes Linking these results to ultimate livestock production phenotypes is still an issue. Repeated traits, such as litter size show similarities between observations beyond the additive genetic effect; this is called permanent environment effect. This can go back to early life experiences, intra-uterine-, lactation- (e.g. colostrum), or vaccination -effects. Partially this relates to Inter- or trans-generational epigenetic effects. The challenge is to properly separate the effects into nutritional, genetic and nutrigenetic origins. Mal- or under-nutrition has clear negative effects on development. Wang et al. (2025) impose maternal feed restriction and correct that through a supplementation of a methylation pathway. It is unclear whether this restores the original phenotype. Araujo et al. (2025) applied methodology to estimate tentative transgenerational epigenetic effects for performance and reproduction traits. For backfat a considerable effect was estimated with a high reset percentage, in layman terms: parental environment influences the backfat of the offspring, this effect wears out quickly over the generations. Quantitative study of phenotypes can indicate possible epigenetic effects. If of sufficient magnitude the combination of quantitative estimates with intermediate phenotypes can make this field develop much faster.

References

Araujo, A.C., Johnson, J.S., Graham, J.R., Howard, J., Huang, Y., Oliveira, H.R., Brito, L.F., 2025. Frontiers in Genetics 15, 1526473. https://doi.org/10.3389/fgene.2024.1526473
Wang, F., Wang, X., Odle, J., Maltecca, C., Lin, X., 2025. The Journal of Nutrition 155, 804-816. https://doi.org/10.1016/j.tjnut.2025.01.012

Trypsin inhibitor proteins regulate gastric acid secretion and intestinal pH homeostasis in pigs

M.J. Nisley[1], E.B. Burrough[2], H.B. Krishnan[3], N.K. Gabler[1]
[1]Iowa State University, Ames, USA. [2]Iowa State University Veterinary Diagnostic Lab, Ames, USA. [3]University of Missouri USDA-ARS, Columbia, USA (mnisley@iastate.edu)

Trypsin inhibitor proteins, inherent in soybeans, attenuate protein digestion and augment pancreatic protease secretions, thereby reducing pig digestion efficiency and lean growth. Less is understood about underlying gene expression and phenotypical modulation related to luminal acid-base balance occurring in the digestive tract of pigs fed high trypsin inhibitor unit (TIU) diets. Therefore, the objective of this research was to evaluate how dietary soybean-derived active TIU concentration influences protein digestion, regional luminal pH, and the expression of gastrointestinal acid-base regulatory genes in late-stage nursery pigs. A total of 48 mixed-sex pigs (5.6 ± 0.90 kg BW) were housed in 16 pens and randomly assigned to one of two dietary treatments (n=8/trt): 1) a low TIU corn-soybean meal-based control diet (CON, 0.87 TIU/mg), or 2) a high TIU corn-soybean meal-based diet (HIGH, 4.29 TIU/mg). Pigs were fed *ad libitum* for 35 d. On d 35, one pig per pen was fasted overnight, then given *ad libitum* access to feed for 30 min. Refusals were collected and weighed to determine individual meal intake. Pigs were then sacrificed 240 min after feed removal to collect gastrointestinal tissue samples and postprandial luminal contents. Apparent nitrogen digestibility coefficients were determined using titanium dioxide as an inert marker in stomach digesta, ileal digesta, and fecal samples. Stomach, ileum, cecum, and colon digesta were analyzed for pH. Fundic stomach tissue, duodenal tissue with the attached pancreas, and ileal tissue were collected and flash-frozen to assess acid-base regulatory gene expression. Data were analyzed using a t-test with the pig as the experimental unit. Over the 35-d trial, CON pigs observed increased weight gain (0.46 vs. 0.36 kg/d; P=0.003) and final BW (21.6 vs. 18.0 kg; P=0.007) compared to HIGH pigs. Apparent stomach nitrogen digestibility was similar between CON and HIGH pigs (-3.88 vs. -5.07%; P>0.10). CON pigs tended to have higher apparent ileal nitrogen digestibility than HIGH pigs (71.3 vs. 55.6%; P=0.067), while apparent total tract digestibility was similar across CON and HIGH pigs (76.7 vs. 74.5%; P>0.10). HIGH pigs had lower stomach pH than CON pigs (2.16 vs. 2.87; P=0.033). Ileal pH showed a trend toward being lower in CON pigs (6.13 vs. 6.45; P=0.091), while cecal pH remained unchanged (P>0.10). Colonic pH was significantly higher in CON pigs (6.25 vs. 5.86; P=0.040). HIGH pigs had a 2.9-fold increase in stomach *ATP4A* gene expression (P=0.044), 5.1-fold increase in stomach *HDC* gene expression (P<0.001), and an 8.7-fold increase in stomach *HRH2* (P=0.003) compared to CON pigs. Duodenal *CFTR* expression increased 4.6-fold in HIGH pigs (P=0.003), while ileal expression remained unchanged (P>0.10). In conclusion, pigs fed a high TIU diets exhibited attenuated ileal protein digestion, increased gastric acid production, and increased acid-buffering activity in the proximal small intestine.

Funding
This project was partially supported by the United Soy Board.

There is more than methane and methionine to one-carbon metabolism

J. van Milgen
PEGASE, INRAE, Institut Agro, Saint-Gilles, France (jaap.vanmilgen@inrae.fr)

Through methane, there is considerable interest in ruminal one-carbon metabolism. However, one-carbon metabolism is also important for the host (i.e., ruminants and non-ruminants), and there are similarities between methanogenesis in the rumen and one-carbon metabolism in the host. Cytosine and adenine residues in DNA and RNA can be methylated by methionine and this methylation affects transcription and gene silencing in DNA and translation in RNA. Modifications in the methylation (and demethylation) of DNA are involved in the development of cancer. Histones are nucleic proteins rich in arginine and lysine around which DNA is wrapped. The methylation of arginine and lysine acids affects the functioning of the histones, including gene expression. Also other proteins can be undergo post-translational modifications (e.g., methylation of histidine in myosin and actin in the muscle). Although methylation and demethylation occur in DNA, this seems to be less or not the case for other methylated metabolites (e.g., methylated histidine from muscle proteins), and many of these are excreted in the urine. The importance of methylation by methionine makes it more than "just an essential amino acid". Once methionine has donated its methyl group to a receptor, the resulting homocysteine can be catabolized further by donating its sulfur to serine yielding cysteine, or by methylation of homocysteine back to methionine (i.e., the methionine salvage pathway). The carbon required for the latter can originate from dietary sources such as choline or betaine, but can also come from other carbon sources metabolized via tetrahydrofolic acid (THF, a vitamin B9 metabolite). The catabolism of tryptophan, histidine, serine, and glycine results in the production of one-carbon moieties. Serine and glycine are unique in this because they can be synthesized de novo. The reversible metabolism of serine to glycine by serine hydroxymethyltransferase (SHMT) releases a one-carbon moiety, and the further catabolism of glycine releases another one-carbon moiety. Because serine can be synthesized from glucose, four of the six carbons from glucose can be retained in one-carbon moieties. Glycine may play a central role in the metabolism of one-carbon moieties because it can either donate a one-carbon moiety via its catabolism or it can accept one through the reversible reaction of SHMT to yield serine (which can be metabolized further to pyruvate). Glycine thus has the potential to act as a one-carbon buffer. Plasma glycine concentrations are typically among the highest compared to those other free amino acids, which could support the hypothesis of the role of glycine as a one-carbon buffer. The simplest organic compounds (i.e., one-carbon moieties) and the simplest amino acid (i.e., glycine) apparently play very important roles in the regulation of metabolism.

Influence of maternal high-forage versus high-concentrate diets during gestation on gas exchange, intake, and growth in finishing beef steers

C.A. Finck, P. Puccetti, J.S. Caton, C.L. Dahlen, K.K. Sedivec, S. Amat, K.C. Swanson
North Dakota State University, Fargo, USA (kendall.swanson@ndsu.edu)

Maternal nutrition during gestation can influence offspring performance through effects on developmental programming, yet long-term effects on growth efficiency in finishing steers remain unclear. Nutrient availability during gestation influences fetal organ development which may result in differences in metabolic programming and postnatal growth efficiency. This study evaluates the impact of feeding high-forage vs. high-concentrate diets to pregnant heifers on offspring growth performance, methane (CH4) and carbon dioxide (CO2) production, oxygen (O2) use, respiratory quotient (RQ), body weight (BW), and dry matter intake during the finishing phase. Heifers (n = 41) predominately of Angus breeding were blocked by initial BW and randomly assigned individually to either feeding a high-forage (75% forage) or high concentrate (75% concentrate) diet using an Insentec feeding system to gain 0.45 kg/day from 15 days before breeding until parturition. Heifers were bred using artificial insemination with male sexed semen from the same sire. Following calving, all cows and calves were managed similarly and after weaning steers were fed backgrounding diets for approximately 3 months before entering the finishing phase. Steers (initial BW ± standard deviation = 293 + 21 kg) were blocked by BW, fed individually using Calan gates, and adapted to a high concentrate diet for two weeks before being placed on a finishing diet for 77 ± 7 days. A subset of steers (n = 22) was selected for a nutrient and energy balance experiment. Feed intake, and fecal and urinary excretion were quantified over a 5-day period and composite gas samples entering and exiting each headbox were analyzed for O2, CO2, and CH4 concentration over a 24-hour period utilizing headboxes. Data were analyzed as a completely randomized block design with treatment (n = 2) and BWblockffects (n = 2 to 4) using the GLM procedure of SAS. Results indicated that treatment had no effect ($P \geq 0.44$) on O2, CO2, CH4, RQ, or DMI. Steers from heifers fed the high-forage maternal diet had greater ($P = 0.007$) BW at the time of the nutrient and energy balance experiment. The greater BW in steers from the heifers fed high-forage diets and the lack of difference in dry matter intake and gas exchange measurements suggest potential differences in metabolic efficiency between treatment groups. Analysis of nutrient and energy digestibility and balance samples should provide further insight on how maternal diet (forage:concentrate) influences efficiency of nutrient and energy utilization in steers fed finishing diets. Funding: USDA-NIFA-AFRI-2022-37092

Funding
USDA-NIFA-AFRI-2022-37092

High starch diet early in life affects hydrolytic activity in the rumen later in life

M. Jamrogiewicz[1], M. Przybyło[1], J. Kański[1], J. Flaga[1], D. Wojtysiak[1], M.P. Majewska[2], U. Wolska-Świętlicka[2], R. Miltko[2], M. Clauss[3], P. Górka[1,4]
[1]University of Agriculture in Kraków, Kraków, Poland. [2]The Kielanowski Institute of Animal Physiology and Nutrition, Polish Academy of Sciences, Jabłonna, Poland. [3]Vetsuisse Faculty, University of Zurich, Zurich, Switzerland. [4]National Research Institute of Animal Production, Balice, Poland (michal.jamrogiewicz@student.urk.edu.pl)

We aimed to assess the long-term effects of a high-starch diet during the first weeks of life on hydrolytic activity in the rumen. Fifty-four male lambs (5–15 days of age; collected in 3 batches) were randomly divided into a control (C) and a high-starch (HS) group, kept in eight group pens (6-7 animals/pen; 4 pens/treatment) and fed ad libitum milk replacer (MR), and either a meadow and alfalfa hay mixture (1:1; C; n=27) or a concentrate mixture (70% barley, 15% wheat bran, 10% soybean meal, 5% chopped hay; HS; n=27). The MR was provided for 7 weeks, followed by solid feeds for 2 weeks after weaning (Phase 1; P1). From week 10, all lambs were transitioned to a hay-based diet and fed ad libitum until 7 months of age (Phase 2; P2). Subsequently, animals were challenged with high-starch diet for 7 days (Phase 3; P3) by replacing 50% of voluntary dry matter consumed hay with barley. At the end of each phase, a subset of 8 (P1/P2) and 11 (P3) randomly selected animals/treatment was slaughtered 3 h after feeding, and reticulorumen digesta samples were collected for analysis, including amylolytic (AA), cellulolytic (CA), xylanolytic (XA), inulinolytic (IA), and pectinolytic activity (PA). Data were analyzed for each phase using a mixed model procedure in SAS. Statistical model included fixed effect of treatment and random effects of animal batch and pen within a batch. In P1, AA was higher in the HS group (9.64 vs. 24.7 uM glucose/g DM/min, SE=5.873; $P=0.05$), while XA was higher in the C group (13.3 vs. 5.20, µM xylose/g DM/min, SE=2.489; $P=0.04$). In P2, AA (11.1 vs. 7.61, SE=1.761) and CA (14.6 vs. 6.48 µM glucose/g DM/min, SE=1462) were higher in the C group ($P \leq 0.03$). In P3, CA tended to be higher in the C group (11.9 vs. 7.62, SE=1.41; $P=0.06$), while no significant differences were found for other analyzed enzymes (AA: 34.5 vs. 28.7, SE=4.95; XA: 15.6 vs. 8.72, SE=5.136; IA: 5.56 vs. 5.48 µM fructose/g DM/min, SE=0.755; PA: 4.83 vs. 4.86 µM glucuronic acid/g DM/min, SE=0.811; $P \geq 0.24$). In conclusion, a high-starch diet early in life influenced hydrolytic activity in the rumen by increasing AA in the first weeks of life but resulting in a reduced XA at this time. Furthermore, the diet fed in first weeks of life affected response in terms of CA in the rumen later in life, both on a hay diet and when a high-starch diet challenge was presented. These findings suggest that early-life dietary interventions can have lasting effects on rumen microbial function, potentially influencing rumen functions in adulthood.

Funding

This research was funded by the National Science Centre, Poland, under the OPUS call in the Weave programme (project No. 2021/43/I/NZ9/02222).

Impact of heat stress on post-translational histone modifications in the liver of Holstein dairy cows

S.M. Sarkar[1], M. Völker-Albert[2], B. Kuhla[1], F. Koch[1]
[1]Research Institute for Farm Animal Biology, Dummerstorf, Germany. [2]MOLEQLAR Analytics GmbH, München, Germany (koch@fbn-dummerstorf.de)

Epigenetic modifications link environmental changes to physiological responses in animals. High ambient temperatures may also be an important driver of epigenetic regulation for a rapid stress response, altering, among others, chromatin accessibility and thus transcription. Histones are crucial for chromatin packing, however, little is known about histone modifications in heat-stressed cattle. Therefore, our objective was to elucidate the effect of 7 days of heat stress on the methylation and acetylation pattern of histones in the liver of dairy cows. Thirty primiparous, non-pregnant German Holstein cows (169 ± 9 days in milk) were allocated to a heat-stressed (HS, n = 10), control (CON, n = 10) or pair-feeding (PF, n = 10) group. During the adaptation phase, all animals were kept in a climate chamber at thermoneutral conditions (16°C; 63% relative humidity (RH), temperature-humidity-index (THI) 60) for 6 days and received a TMR twice daily. During the experimental phase, HS cows were exposed to 28°C (52% RH, THI = 76) and CON cows exposed to 16°C (63% RH, THI = 60) for 7 days, with *ad libitum* feeding. PF cows were exposed to identical environmental conditions as CON, but were offered only the amount of feed the HS cows ingested. After 7 days of treatment, liver samples were taken and frozen at -80°C for further analysis. Histones were isolated by acidic extraction and methylation and acetylation of histone 3 and 4 were detected by LC-MS/MS analysis. Data were analyzed with ANOVA using the MIXED procedure of SAS (Version 9.4) with the fixed effects of treatment (HS, CON, PF), block (1-10), and their interactions; and days in milk served as a covariate. Multiple comparisons were tested with a Tukey-Kramer test. The acetylation of H3K4, H3K23, H4K5, H4K8, H4K12, and H4K16 were higher in HS and CON than PF cows, whereas the acetylation of H3.3K36 was higher in HS and PF than CON animals ($P < 0.05$, respectively). Furthermore, the mono-methylation of H4K20 was higher in HS than CON and PF cows ($P < 0.05$). However, the mono-methylation of H3K4 and H3K9 was higher in HS and CON than PF cows ($P < 0.05$, respectively). Di-methylation of H4K20 and tri-methylation of H3K9 was lower in HS than PF but comparable between HS and CON cows, whereas the di-methylation of H4K20 was lower in CON than PF cows ($P < 0.05$, respectively). In conclusion, the main driver of the changes in histone acetylation and methylation was the reduction in feed intake. High ambient temperatures but not pair-feeding altered H4K20 mono-methylation, suggesting its involvement in chromatin accessibility and DNA replication (Corvalan et al., 2021) during heat acclimation. Future research is needed to link the post-translational histone modifications with the metabolic responses of the liver.

Reference

Corvalan, A.Z., Coller, H.A., 2021. Physiological Genomics 53, 22-32.
https://doi.org/10.1152/physiolgenomics.00128.2020

Funding

This work was funded by the German research foundation (grant number KO 5972/3-1).

High starch diet early in life hardly affect rumen morphology parameters later in life

M. Jamrogiewicz[1], A. Garus-Piętak[1], M. Przybyło[1], J. Kański[1], J. Flaga[1], D. Wojtysiak[1], S. Świerk[1], R. Miltko[2], M. Clauss[3], P. Górka[1,4]

[1]1University of Agriculture in Kraków, Kraków, Poland. [2]2The Kielanowski Institute of Animal Physiology and Nutrition, Polish Academy of Sciences, Jabłonna, Poland. [3]3Vetsuisse Faculty, University of Zurich, Zurich, Switzerland. [4]4National Research Institute of Animal Production, Balice, Poland (aleksandra.garus-pietak@student.urk.edu.pl)

We aimed to determine the long-term effect of a high starch diet in the first weeks of life on rumen morphology parameters. Fifty-four male lambs (5-15 days of age; collected in 3 batches) were randomly divided into a control (C) and a high-starch (HS) group, kept in eight group pens (6-7 animals/pen) and fed ad libitum milk replacer (MR), and either meadow and alfalfa hay (1:1; C; n=27) or a concentrate mixture (70% barley, 15% wheat bran, 10% soybean meal, 5% chopped hay; HS; n=27). The MR was provided for 7 weeks, followed by solid feeds for 2 weeks after weaning. From week 10, all lambs were transitioned to a hay-based diet and fed ad libitum until 7 months of age. One week after weaning (Phase 1; P1) and at the age of 7 months (Phase 2; P2), 8 animals (P1/P2) were randomly selected/treatment (1-2 animals/pen) and slaughtered 3 h after morning feeding. Rumen tissue samples from atrium, ventral and dorsal sac were collected for microscopic analysis, including papillae length and width, thickness of whole epithelium, stratum corneum and living strata, and number of balloon cells. Twenty-four papillae were measured/animal for gross measurements, whereas epithelium, stratum corneum, living strata and balloon cells were measured on 8 randomly selected papillae. Data were analyzed for each phase with a mixed model procedure in SAS. Statistical model included fixed effect of treatment, and random effects of animal batch and pen within a batch. In P1, papillae width in atrium (457 vs. 353 µm) and dorsal (546 vs. 416 µm) and length in ventral (1725 vs. 1083 µm) and dorsal (1377 vs. 988 µm) were higher for HS ($P \leq 0.03$). Stratum corneum in atrium (50.5 vs. 31.4 µm), ventral (52.9 vs. 31.0 µm) and dorsal (59.6 vs. 27.1 µm), and epithelium in atrium (140 vs. 115 µm) and dorsal (157 vs. 112 µm) were thicker for HS ($P \leq 0.04$). Moreover, living strata in dorsal tended to be greater for HS (97.3 vs. 84.6µm; $P = 0.06$). The number of balloon cells (empty large cells in the stratum corneum) in atrium (2.40 vs. 1.38), ventral (2.32 vs. 1.38) and dorsal (2.29 vs. 1.13) was greater for HS ($P \leq 0.05$). No differences were found in P2, except papillae length in atrium which were higher for C (1786 vs. 1425µm; $P < 0.01$). In conclusion, a high-starch diet early in life stimulated rumen papillae and epithelium development. However, this effect did not persist in the long term, as most morphological parameters did not differ in P2. These findings suggest that early-life exposure to a high-starch diet may temporarily accelerate rumen epithelium development, but these effects may diminish later in life when lambs are transitioned to a hay-based diet.

Funding

This research was funded by the National Science Centre, Poland under the OPUS call in the Weave programme (project No. 2021/43/I/NZ9/02222)

Integrated multi-omics reveals ruminal microbiota–host interaction and its effect on feed efficiency in beef cattle

J. Sun, G.Y. Zhao, M.M. Li
China Agricultural University, Beijing, China (limeng2021@cau.edu.cn)

While microbiota-host interactions are known to regulate nutrient metabolism, the genetic mechanisms underlying their coordinated effects on feed efficiency remain poorly characterized in beef cattle. This study investigated the genetic and microbial determinants of residual feed intake (RFI) in growing beef cattle by integrating genome-wide association study (GWAS), microbial GWAS (mGWAS), and metagenomic analyses. We aimed to unravel how ruminal microbiota-host crosstalk shapes feed efficiency. A cohort of 100 beef cattle fed the same diet for 90 days was phenotyped for 21 traits spanning growth performance, feed efficiency, nutrient digestibility, and ruminal fermentation. Single-trait GWAS identified 24 significant SNPs associated with RFI, including loci near *ADAMTS7*, *TBC1D2B*, and *POU4F3*, which are linked to metabolic regulation and nutrient sensing. Multi-trait GWAS revealed 42 loci ($P < 1 \times 10^{-6}$) associated with nutrient digestibility traits (crude protein, neutral detergent fiber, and acid detergent fiber digestibility), enriched in pathways regulating amino acid starvation response, mTORC1 signaling, and carbohydrate metabolism. Heritability analysis identified 44 bacterial genera with moderate to high heritability (0.05–0.95), including *Prevotella* and *Fibrobacter*, which are key players in ruminal fermentation and nutrient digestibility. Integration of mGWAS and metagenomic highlighted 4 core genera (*Fibrobacter*, *Alkaliphilus*, *Bacillus*, and *Prevotella*) consistently linked to high abundance, heritability, and network centrality, with significant correlations to RFI and nutrient digestibility. Functional profiling of metagenome-assembled genomes (MAGs) from RFI-associated MAGs, such as *UBA2804*, uncovered in amino acid biosynthesis and carbon metabolism. Structural equation modeling (SEM) demonstrated that RFI is collectively shaped by nutrient digestibility, microbial diversity, and rumen fermentation. Host genetic variants, particularly SNPs in *PRKAR2B* and *RIMBP2*, modulate RFI-related traits by influencing microbial community structure and function. These findings reveal dual mechanisms with host genetics shaping fat metabolism and immune costs and microbiota optimizing fiber degradation and energy harvesting. This integrative framework provides actionable targets for improving feed efficiency through genetic selection and microbial interventions.

Funding
This work was funded by National Key R&D Program of China (2022YFA1304201).

The effect of thermal treatment of field peas on weaned piglets' nutrient digestibility, short chain fatty acids concentration and goblet cell distribution

J. Schulze Holthausen, J. Zentek
Institute of Animal Nutrition, Department of Veterinary Medicine, Freie Universität Berlin, Berlin, Germany (johannes.holthausen@fu-berlin.de)

Field pees seem to be a suitable alternative to replace soybean meal as a protein source in diets for weaned piglets. Nevertheless, the effects of thermal treatment of field peas in weaned piglets are inconclusive. This study aimed to investigate the effect of thermal treatment on peas fed to weaned piglets with special regards to their effects on praecaecal nutrient digestibility and short chain fatty acid (SCFA) concentration in jejunum and colon of weaned piglets. In total 48 piglets, were weaned at 35 d of life and fed isonitrogenous and isoenergetic weaning diets containing either 20 % grounded peas either untreated (Pea), steamed at 85°C for 10 min (Pea-S), autoclaved at 110°C, 15 min (Pea-A) or soybean meal (Con). After two weeks 24 male piglets were euthanized, ileal and colonic digesta, faeces as well as jejunal and colonic tissues were sampled for further analysis. AB-PAS staining was used to quantify goblet cells with the help of QuPath (Version 0.5.1). For statistical analysis a univariate ANOVA was used. $P \leq 0.05$ indicated significant results and $P < 0.01$, trends. Apparent praecaecal digestibility (pcd) of crude protein ($P = 0.073$) was highest in weaned pigs fed Pea-A diets. No impact on the prececal digestibility (pcd) of starch or the apparent total tract digestibility (ATTD) of crude protein and starch was observed. A trend for a higher crude fibre ATTD was visible for Pea-A compared to Con diets ($P = 0.055$). Isovaleric acid concentration tended to be highest in ileum digesta of piglets fed with Pea diets ($P = 0.062$). Concentration of acetic acid in ileum was numerically the highest in the ileum of piglets fed with Pea-A ($P = 0.348$) compared to the other experimental diets. No effect on the number of goblet cells in jejunum or colon was visible. Results demonstrate that thermal treatment of field peas included in diets of weaned piglets at a level of 20% may affect crude protein and crude fibre digestibility with little effects on the SCFA in the ileum.

Local legumes as a sustainable alternative to soya in growing Landrace × Large-White pig diets

A. Román, I. Fernández-Fígares, M. Lachica, C. García-Contreras, R. Nieto
Estación Experimental del Zaidín, Granada, Spain (a.roman@eez.csic.es)

Climate change is a global challenge, and pig production and the European Union's reliance on imported soybean meal as a protein source for feeds exacerbates the problem (Yang et al., 2023 & Tallentire et al., 2018). Our aim was to evaluate local protein sources as an alternative to soya through their impact on pig performance, nutrient fluxes, plasma metabolites and markers of intestinal inflammation. A total of thirty-two Landrace × Large-White pigs were randomly allocated in body weight blocks into four experimental groups: a control group fed diets including soybean meal and three groups in which 50% of the protein provided by soybean was partially replaced by local legume protein (peas, chickpeas and lentils, the last two from waste). Diets were isoproteic and isocaloric, covering animals' needs and formulated according to the ideal protein concept. Pigs were individually housed under temperature-controlled conditions (25 ± 2°C and 21 ± 2°C, respectively, at the beginning and the end of the trial) with access to water and feed ad libitum. Pigs were weighed weekly. The initial weight was 14 ± 0.3 kg and the final weight 66 ± 0.9 kg. After 3 weeks, faecal digestibility and nitrogen balance were assessed in 24 animals (6 per treatment). Samples of faeces and urine were obtained quantitatively for 5 days to determine dry matter, organic matter, nitrogen and energy ingested and excreted. After 10 weeks, pigs were slaughtered and blood samples were taken to analyse biochemical parameters: albumin, β-hydroxybutyrate, HDL-cholesterol, LDL-cholesterol, total cholesterol, creatine, glucose, lactate, triglycerides and urea. At the end of the study samples of faeces were collected for biochemical analysis for markers of intestinal inflammation: calprotectin and myeloperoxidase. An analysis of variance was used to assess treatment effects, with a significance level of 0.05. No significant differences were found among treatments for average daily gain, feed intake, feed efficiency, plasma biochemical parameters and markers of intestinal inflammation ($P > 0.05$). Likewise, there were no differences in apparent faecal digestibility of nutrients and energy. The average nitrogen retention was 23.5 g/day, with no differences between treatments, although both the retained nitrogen/ingested nitrogen and retained nitrogen/digestible nitrogen ratios were higher in the local legume treatments compared to the control (10% and 9%, respectively) ($P < 0.05$), which may indicate a higher nitrogen utilisation efficiency in pigs fed local legumes. Partial substitution of soy protein by local legumes did not negatively affect performance, plasma parameters, markers of intestinal inflammation, nutrient digestibility or nitrogen balance in pigs. These results suggest that the use of local legumes could improve the sustainability of pig production chain.

References

Tallentire, C.W., Mackenzie, S.G., Kyriazakis, I., 2018. Journal of Cleaner Production 187, 338-347. https://doi.org/10.1016/j.jclepro.2018.03.212
Yang, P., Yu, M., Ma, X., Deng, D., 2023. Foods 12, 3423. https://doi.org/10.3390/foods12234203

Funding

Funded by the EU project Facilitating Innovations for Resilient Livestock Farming Systems, abbreviation: Re-Livestock (reference number: 101059609).

Effects of a multi-carbohydrase supplementation on digestive and metabolic utilization of energy in growing pigs

P. Cozannet[1], F.A. Eugenio[1], T. Mahmood[1], M. Jlali[1], J. Noblet[2]
[1]Adisseo, Saint Fons, France. [2]ex INRAE, Rennes, France (Pierre.Cozannet@adisseo.com)

Net energy (NE) evaluation of feeds offers significant benefits for exogenous enzyme assessment in monogastrics nutrition. This study investigates the effect of a multi-carbohydrase (MC) on digestible (DE), metabolizable (ME) and NE in growing-finishing pigs fed a wheat-barley-peas-soybean meal-based diet. Six replicates of 2 littermate pigs with initial body weight of 48-50 kg were used. In each replicate, one pig received the control diet without MC (C diet) and the other one the control diet supplemented with MC (MC diet). The experiment was split into an 11 days adaptation period followed by a 10 days measurement period in a respiration chamber. Animals were provided 2 kg of feed per day, distributed in 3 meals. Feed intake, fecal output and heat production (HP) and its components were measured. The apparent total tract digestibility (ATTD) of energy was not significantly improved (+0.7% unit) and the ME:DE ratio was slightly lower (-0.2% unit; $P > 0.05$) with MC supplementation. A detailed analysis of HP and its components showed that HP, adjusted for similar levels of physical activity and feed intake, tended to be lower in pigs fed the MC diet, leading to an increase of retained energy (+32 kJ/day/kg $BW^{0.60}$, $P < 0.05$) and the efficiency of ME for NE (78.6 vs 77.6%, $P < 0.05$) and then a reduced thermic effect of feeding (-30 kJ/day/kg $BW^{0.60}$, $P < 0.05$) in MC diet. Consequently, the ME and NE contents of MC diet were 0.12 and 0.23 MJ/kg DM ($P < 0.05$) higher. The present study suggests a better and higher evaluation of MC effect on energy value in a NE system compared with ME or DE systems in connection with a better account of the metabolic improvement associated with MC supplementation. Further work is required to identify the origin of energy saving associated with MC supplementation.

A Nutraceutical Compound from Algae and Legumes Accelerates Weight Loss in Obese Pig Models

C. Garcia-Contreras[1], R. Martínes[2], A. García-Beltrán[2], A. Guzmán[2], M. López-Juardo[2], J.M. Porres[2], R. Nieto[1], M. Lachia[1], I. Fernández-Figares[1]
[1]1Department of Nutrition and Sustainable Animal Production, Estación Experimental del Zaidín, CSIC, Granada, Spain. [2]2Department of Physiology, Institute of Nutrition and Food Technology (INyTA), Biomedical Research Centre (CIBM), Sport and Health Research Institute (IMUDS), University of Granada, Granada, Spain (consolacion.garcia@eez.csic.es)

The incidence of obesity and metabolic diseases is growing dramatically in Western and rapidly developing countries. Key causes include postnatal exposure to obesogenic lifestyle, prenatal programming by undernutrition and intrinsic ethnic features with development of a "*thrifty genotype"* for adapting to food scarcity. The Iberian pig is an amenable model for studies in obesity and associated diseases, due to its *thrifty genotype* and a polymorphism in the leptin receptor (LEPR) at the hypothalamic level causing leptin resistance and high appetite levels. Dietary interventions combined with bioactive compounds could be promising strategies for body weight (BW) and fat loss in obese individuals. This study evaluated the effects of a nutraceutical supplement derived from algae and legumes on BW and fat accumulation in an obese pig model. Twenty-four pure Iberian castrate male pigs (44 ± 5 kg BW) were assigned to 2 groups until week 8: a control group (C, n=8) fed a standard diet restricted to 70% of *ad libitum* feeding; and an obese group (OB, n=16) fed *ad libitum* an obesogenic diet (+ 3% extra fat). From week 8 to the end of trial (week 15), group C remained on the restricted standard diet, while the OB group was divided into two groups: an OB-STD group (n=8) fed the hypercaloric diet but restricted to 70% *ad libitum*; and an OB-NUT group (n=8) following the same restriction but supplemented with 0.5g/kg of the nutraceutical. Weekly, individual BW was measured, feed intake was adjusted and average daily gain (ADG) was calculated. Back-fat thickness was measured biweekly using ultrasonography starting at week 8. Linear measurements, including thoracic and abdominal circumference, and body length, were measured at the beginning and end of the trial. The obesogenic diet significantly increased ADG from week 3 ($P < 0.001$) while feed intake augmented in the OB group from the beginning ($P < 0.05$). At week 8, no significant differences were observed in ADG or BW between OB and C groups. However, from week 8 to week 15, weight gain in the OB-NUT group was lower than in the OB-STD and C group (0.463, 0.523 and 0.692 g/day for OB-NUT, OB-STD and C group respectively; $P < 0.001$). The obesogenic diet increased back-fat thickness until week 9 ($P < 0.05$), but, after the start of restriction and nutraceutical supplementation, a reduction in back-fat was observed. Thus, at week 15, the back-fat thickness was lower in OB-NUT compared to C group ($P < 0.01$). No significant differences were found between groups for any of the linear measurements. In conclusion, the findings support the potential of algae and legume-based nutraceuticals as an adjunct to dietary restriction for obesity management. Further research is needed to elucidate the underlying mechanisms and assess translational applications in human populations.

Funding

Funded by FEDER/Junta de Andalucía-Consejería de Transformación Económica, Industria, Conocimiento y Universidad (B-AGR-662-UGR20).

Effect of pelleting on the energy utilization of wheat and barley-based diets in growing pigs

E. Labussiere[1], A. Agouros[2], M. Le Gall[2], K. Quemeneur[2], Y. Lechevestrier[2], N. Quiniou[3]
[1]PEGASE, INRAE, Saint-Gilles, France. [2]Provimi, Crevin, France. [3]IFIP - Institut du Porc, Pacé, France (etienne.labussiere@inrae.fr)

Feed is commonly pelleted in feed mills but without considering consequences on the digestive and metabolic use of energy by growing pigs and on the nutritional values of diets. The objective of this study was to determine effects of pelleting on faecal digestibility and metabolic use of energy in growing pigs when diets were formulated using wheat and barley, alone or mixed, as main ingredients. The study was divided in two experiments. In the first experiment (mean body weight of the pigs: 52.2 kg; n=21), two diets (1 g of SID lysine to net energy –NE) were formulated with either 72% of wheat or 77% of barley, with soybean meal as protein source. In the second experiment (mean body weight of the pigs: 81.9 kg; n=24), two diets (0.85 g of SID lysine to net energy –NE) were formulated with either 73% of wheat or a mixture of 39% of wheat and 39% of barley. In all experiments, the diets were fed as mash or pellets resulting in 4 dietary treatments per experiment. The pigs were restrictedly fed at 1574 or 2347 g DM/d for experiments 1 and 2, respectively. After adaptation to their diets, the pigs were housed in an open-circuit respiration chamber to measure nitrogen and energy balance with continuous measurements of total heat production (HP) during six days, and the fasting HP during a seventh day when the pigs did not receive any feed. The digestibility of energy of the diets was significantly increased because of pelleting (+2.6 and +1.2% for wheat and barley-based diets in experiment 1; +2.1 and +2.5% for the wheat-based diet and the mixture of barley and wheat-based diet respectively; $P<0.01$). In the first experiment, pelleting resulted in a decrease in daily protein deposition rate (-9%; $P=0.03$), because of asynchronous absorption of amino acids and glucose (Agouros et al., 2023) but total HP was not affected. In the second experiment, daily protein deposition was not affected because of pelleting but total HP increased with the pelleted wheat-based diet. Pelleting did not significantly affect the fasting HP, but values were consistently 3 to 7% lower with the pelleted diets than with the non-pelleted diets. In the first experiment, the NE value of diets was not improved by pelleting because of the decreased protein deposition. In the second experiment, pelleting resulted in a significant increase of NE values (+ 4% on average for the two diets; $P<0.01$). The continuous measurements of HP indicated that the post-prandial increase of HP was delayed because of pelleting, suggesting that pelleting may also change the metabolic pathways involved in nutrient use.

Reference

Agouros A., Le Gall M., Quemeneur K., Lechevestrier Y., Montagne L., Quiniou N., Labussière E., 2023. Pelleting and botanical source influence starch utilization in growing pigs. In: Proc. of the 74th EAAP annual meeting, Lyon, France, 209.

Funding

Provimi France – Cargill (Crevin, France), financially supported this work.

A combination of a multi-strain Bacillus spp. direct-fed microbial and a protease improves growth performance, carcass value and positively shifts fecal microbiome of grow-finishing pigs

E. Vinyeta[1], P. Aymerich[1], D.E. Velayudhan[1], Q. Wang[2], J. Remus[3]
[1]Danisco Animal Nutrition & Health (IFF), Oegstgeest, Netherlands. [2]Danisco Animal Nutrition & Health (IFF), Wilmington, USA. [3]Danisco Animal Nutrition & Health (IFF), Wilmington, Netherlands (ester.vinyeta@iff.com)

A combination of three *Bacillus* spp. direct feed microbial with a protease (DFM+PRO) has shown major structural fiber breakdown and protein solubilization of fiber-rich diets leading to an increased energy and nitrogen digestibility and improved performance in growing pigs. In addition, the *Bacillus* DFM has shown probiotic properties related to gut health (Payling et al., 2017). This study evaluated the effects of DFM+PRO blend on growth performance, carcass characteristics and microbiota composition of grow-finishing pigs. A total of 1,056 pigs (Fast 276 x PIC 800, initial body weight 30.7 kg) were allocated into 3 dietary treatments with 16 replicates (22 pigs/pen, with 1:1 immunologically castrated males and females placed in separate pens). Diets were based on corn (>50%) and supplied as mash in 5-feeding phases. Treatments comprised: PC) commercially relevant nutritional specs, with Net Energy (NE) of 2500 kcal/kg constant through all phases and standardized ileal digestible lysine (SID Lys) reduced over age from 1.04 to 0.67%; NC) negative control with SID Lys and NE reduced from the PC standards (-0.036% and -25 kcal/kg, respectively); DFM+PRO: NC supplemented with 3-strain of *Bacillus* spp. each at 1.5×10^8 CFU/kg feed and protease at 5,000 U/kg. Pig pen weight was recorded on day 1 and every 3 weeks until week 15 (end of trial). Average daily gain (ADG), average daily feed intake and feed conversion ratio (FCR) were calculated per pen for each phase. Days to market were measured and carcass characteristics were evaluated at the slaughterhouse. Rectal samples were collected (10/treatment) at week 1, 3, 6, 9 and 12 of trial and Illumina 16S amplicon sequencing was performed using 16S V4 primer. Data was analyzed in a linear model including the effects of treatment, room and gender. The NC diet did not impact growth performance except for an increased FCR in the first phase ($P<0.05$) and a greater ADG in the last phase ($P<0.05$) compared to PC. The supplementation of DFM+PRO tended to reduce the days to market (-1.2 days) compared to NC ($P=0.06$) and increased carcass weight compared to the PC (112.0 vs. 110.1 kg; $P<0.05$), reduced belly fat percentage compared to PC and NC ($P<0.01$) and tended to increase marbling compared to PC and NC ($P=0.06$). The 16S microbiome analysis revealed that at week 1 supplementation of DFM+PRO increased beneficial bacteria (*Turicibacter*, *Rombustia*; $P<0.05$) and reduced pathogenic bacteria (*Streptococcus* spp.; $P<0.05$) compared to NC. At week 9 DFM+PRO supplementation significantly reduced *Streptococcus* and *Methanobrevibacter* ($P<0.05$). In conclusion, a combination of a multi-strain *Bacillus* spp. direct-fed microbial, and protease reduced days to market, improved carcass weight and characteristics and promoted positive shift on gut microbiota of grow-finishing pigs.

Reference

Payling, L., Kim, I.H., Walsh, M.C., Kiarie, E., 2017. Journal of Animal Science 95, 4017-4029. https://doi.org/10.2527/jas.2017.1522

Effect of a consensus bacterial 6-phytase variant on phosphorus digestibility and reproductive performance in sows from 3 studies

D.E. Velayudhan[1], X. Benthem de Grave[2], G. Dusel[3], E. Vinyeta[1], P. Aymerich[1], Y. Dersjant-Li[1]
[1]Danisco Animal Nutrition & Health (IFF), Oegstgeest, Netherlands. [2]Schothorst Feed Research B.V, Lelystad, Netherlands. [3]University of Applied Sciences Bingen, Bingen, Germany (Deepak.Evelayudhan@iff.com)

This study assessed a bacterial 6-phytase variant (PhyG) on apparent total tract digestibility (ATTD) of calcium (Ca) and phosphorus (P) in gestating (Exp. 1) and lactating sows (Exp. 2), and on reproductive performance over two reproductive cycles (Exp. 3). In Exp. 1, 40 sows (parity 2-6) at day 78 of gestation; and in Exp. 2, 40 sows (parity 2-6) at day 4 of lactation were randomly assigned to one of three diets. Both experiments included a positive control (PC) diet with adequate levels of Ca and ATTD P (0.65 and 0.25%, and 0.70 and 0.32% for gestation and lactation diets, respectively), negative control (NC) diet with low Ca and ATTD P levels (0.50 and 0.10%, and 0.50 and 0.15% for gestation and lactation diets, respectively), and NC supplemented with PhyG (500 FTU/kg). Diets included corn, soybean meal, and sunflower meal (phytate P: 3.0 g/kg) with restricted feeding during gestation and ad libitum feeding during lactation. After 14-day adaptation, fecal samples were collected over 4 days to determine ATTD of Ca and P. In Exp. 3, 60 sows (parity 3.1 ± 0.4, initial BW 211.1 ± 11.1 kg at insemination) were fed either PC diet adequate in net energy (NE: 2127 and 2342 kcal/kg for gestation and lactation, respectively) and nutrients (0.63 and 0.91% standardized ileal digestible Lys, 0.75 and 0.95% Ca, and 0.24 and 0.27% standardized total tract digestible P for gestation and lactation, respectively) or without added inorganic phosphate, nutrient-reduced (0.65 and 0.76% Ca for gestation and lactation, respectively, -54 kcal/kg NE [for lactation only], and -0.02% points standardized ileal digestible Lys [for lactation only]) diet supplemented with PhyG (1,000 FTU/kg). Diets contained barley, wheat, corn, soybean and sunflower meal, with feeding restricted during gestation and ad libitum during lactation. Sow reproductive performance and litter growth were monitored across two consecutive reproductive cycles. Data were analyzed using ANOVA, using JMP 16.1. Results showed that PhyG (compared to NC) improved P digestibility (19.8% to 34.5% in gestation; 27.9% to 44.3% in lactation; $P<0.05$) and numerically improved Ca digestibility (17.9% to 22.3% in gestation; 32.1% to 35.4% in lactation). In Exp. 3, PhyG supplementation (compared to PC) maintained sow BW gain during gestation, BW loss until weaning, body condition, backfat thickness, and litter outcomes ($P>0.10$), while reduced days to weaning (25.7 vs 27.9 days) and increased piglet BW gain (270g vs. 229g; $P<0.05$). Application of PhyG matrix resulted in a 6.5% reduction in feed cost. In conclusion, PhyG improved ATTD of P in gestation and lactation sows fed Ca and P-reduced diets. Supplementing an inorganic phosphate-free, energy and nutrient-reduced diet with PhyG maintained sow and litter performance, lowered feed costs, and enhanced production benefits, supporting sustainable sow farming

EU circles project: multi-omics analysis reveals distinct microbial networks linked to a blend of chestnut and quebracho extract supplementation in weaned pigs under different farming conditions

F. Correa[1], D. Luise[1], G. Palladino[2], F. Palumbo[1], D. Schicchitano[2], G. Babbi[2], A. Castagnetti[3], S. Rampelli[2], M. Candela[2], P. L. Martelli[2], P. Trevisi[1]
[1]Department of Agro-Food Sciences and Technologies, University of Bologna, Bologna, Italy. [2]Department of Pharmacy and Biotechnology, University of Bologna, Bologna, Italy. [3]Wellmicro, Bologna, Italy (federico.correa2@unibo.it)

Weaning represents a critical phase in pig production, often marked by physiological and microbial shifts that can impair growth and health. Tannins, recognized for their antimicrobial, antioxidant, and anti-inflammatory properties, hold potential for enhancing gut health. This study explored the impact of a chestnut and quebracho tannin blend (Silvafeed Nutri P/ENC for Swine, Silvateam, Italy) on the growth performance, health status, and fecal metagenomic and metabolomic profiles of weaned pigs raised in two distinct farming environments. A total of 160 pigs (24 ± 2 days old; T0) were assigned to two weaning units classified as high (H) or low (L) performance, based on body weight and litter origin. Within each unit, pigs were divided into two dietary groups: a Control group (CON; 40 pigs) receiving a standard weaning diet, and a Treated group (TRT; 40 pigs) fed the same diet supplemented with 2 g/kg of the tannin blend. Body weights were recorded at days 35 (T1), 49 (T2), and 76 (T3) post-weaning, while fecal samples were collected at the same intervals for metagenomic profiling and metabolomic analysis (Metabolon, Inc. platform). Growth performance was assessed using a linear model that included dietary group (TRT vs. CON) and farming condition (H vs. L), while metabolomic data were analyzed via Sparse Partial Least Squares Discriminant Analysis (sPLS-DA), and microbial species were identified through a multi-kingdom approach. At T2, the diarrhea index was significantly lower in the TRT group compared to the CON group in farm L ($P = 0.02$). Microbial alpha diversity at T2 and T3 was reduced in farm L ($P < 0.05$) and in the TRT group compared to CON ($P < 0.01$). Beta diversity, for both microbial species and functions, was significantly affected by the interaction between farming conditions and dietary group across all time points ($P < 0.05$), suggesting a different microbial composition and functional profile between farm and within each group. At T2, sPLS-DA identified Urolithin B and Isourolithin A—metabolites associated with tannin metabolism—as being linked to the TRT group in farm L. Weighted gene correlation network analysis revealed correlations ($r > 0.5$) identified modules containing bacteria like *Ellagibacter isourolithinifaciens* associated to Urolithin metabolites. In conclusion, supplementation with a dietary tannin blend reduced diarrhea under suboptimal sanitary conditions by promoting a microbial network capable of metabolizing tannins and generating metabolites that support gut health.

Funding

Research supported the European Union's Horizon 2020 (agreement n.818290.) "Controlling Microbiomes Circulations for Better Food Systems" (CIRCLES) project.

Effect and prediction of carbohydrase enzyme supplementation on growth performance of broilers

P. Cozannet[1], R.B. Shirley[2], H. Yakout[2]
[1]Adisseo, Lyon, France. [2]Adisseo, Alpharetta, USA (Pierre.Cozannet@adisseo.com)

A meta-analysis investigated the performance of exogenous enzyme (Rovabio Advance; Adisseo), across 27 trials with 84 diets. The dataset included 2,774 individual data entries covering various strains over 35-to-42-day trial periods. A random-effects model was used for data analysis. The primary objectives were to evaluate the average response of the enzyme on broiler performance, identify the key factors influencing its effectiveness, and explore potential strategies for optimizing feed formulation. Studied parameters were feed intake (FI), feed conversion ratio (FCR) and average daily gain (ADG). Enzyme significantly improved performance of broilers. Average (min/max) were 0.83 (-4.0/4.6) and -1.3 (-6.8/2.3) for ADG and FCR, respectively. The analysis demonstrated that diet composition was the most significant factor affecting enzyme response, explaining approximately 79% of the observed variability. Principal component analysis suggests that the source and level of raw materials and nutrients can explain 21 and 59% of the variability, respectively. Predictive equations were developed for FI, ADG and FCR prediction, based on metabolizable energy, available phosphorus and digestible amino acids, with respective determination coefficients being 0.93, 0.90 and 0.90 for FI ADG and FCR, respectively. The enzyme effect was predicted taking into account soluble arabinoxylan content with determination coefficients of 0.93, 0.95 and 0.93. Taking the soluble arabinoxylan content into account, the predicted enzyme effect on the coefficients on FI, ADG and FCR was 0.93, 0.95 and 0.93, respectively. The findings underscore the value of customizing feed formulations based on specific nutrient compositions and substrate content, enabling more effective utilization of enzymes in poultry nutrition. The results also suggest the need for further research to optimize enzyme application in diets with varying nutrient densities, substrate content and for other feed additives like phytase and protease. Additionally, the potential of using soluble arabinoxylan (AX) content to better predict enzyme response was discussed, along with the need for more precise calibration techniques, such as NIRS, for evaluating dietary AX levels.

Small intestinal starch digestion in cattle receiving postruminal infusion of glycoside hydrolases

R.J. Trotta[1], K.C. Swanson[2], R. Garcia-Gonzalez[3], K.M. Kragh[3], Shukun Yu[3], D.L. Harmon[1]
[1]University of Kentucky, Lexington, USA. [2]North Dakota State University, Fargo, USA. [3]Danisco Animal Nutrition & Health (IFF), Oegstgeest, Netherlands (ronald.trotta@uky.edu)

Under certain experimental conditions, enzymatic digestion of starch and absorption of glucose in the ruminant small intestine can provide more energy to the host compared with ruminal fermentation of starch (Harmon and McLeod, 2001). However, deficient activity of pancreatic α-amylase and/or small intestinal α-glucosidases limits small intestinal starch digestibility and digestible energy available for absorption in ruminants. The objective of the current study was to evaluate the influence of increasing exogenous glycoside hydrolases on small intestinal starch digestibility and ileal nutrient flow in cattle. Six Angus × Holstein steers, surgically fitted with infusion catheters in the abomasum and duodenum and ileal sampling cannulas, were used in three 6 × 6 Latin Square design experiments evaluating three sources of exogenous glycoside hydrolases: one α-amylase and two glucoamylases (GA1 and GA2). Steers were fed an alfalfa cube/pelleted beet pulp diet to minimize dietary starch intake. A solution of raw corn starch containing 5% v/v CrEDTA was continuously infused through abomasal catheters to provide 50 g starch/h. Each enzyme was continuously infused through duodenal infusion catheters (post-pancreatic duct) at six levels: 0 (negative control), 0.0625, 0.125, 0.25, 0.5, or 1 g of enzyme protein per hour. Each experiment was 6-d in length, consisting of six 12-h treatment periods with 12 h for washout between periods. Six ileal digesta samples were collected in hourly intervals, pooled, and then analyzed for DM, Cr, starch, glucose, and enzyme activity. Data were analyzed using SAS for effects of treatment, period, and steer. In Experiment 1, duodenal infusion of exogenous α-amylase did not influence ($P \geq 0.09$) ileal starch concentration, ileal starch flow, or small intestinal starch disappearance. In Experiment 2, duodenal infusion of GA1 linearly decreased ($P < 0.01$) ileal starch concentration and flow, resulting in a linear increase ($P < 0.001$) in small intestinal starch disappearance (51.1% of infused for control to 76.4% of infused for 1 g protein/h). Duodenal infusion of GA1 linearly increased ($P < 0.003$) ileal glucose and glucoamylase concentration and flow. In Experiment 3, duodenal infusion of GA2 linearly increased ($P < 0.001$) ileal glucose and glucoamylase concentration and flow but, small intestinal starch disappearance did not change ($P \geq 0.29$) with duodenal GA2 infusion. The findings of the current study support the hypothesis that small intestinal α-glucosidases are the primary hydrolytic limit of small intestinal starch digestion in cattle. Small intestinal supply of exogenous glucoamylase increased small intestinal starch digestibility up to 50% and glucose energy available for absorption by 1.72 Mcal/d.

Reference

Harmon, D.L., McLeod, K.R., 2001. Journal of Animal Science 79, E59-E72. https://doi.org/10.2527/jas2001.79E-SupplE59x

Funding

This research was supported by Danisco Animal Nutrition & Health, IFF.

Effect of mimosine on thyroid hormones and maintenance energy requirement of cattle

A. Cadenas-Soberanis[1], R. Jiménez-Ocampo[2], J.I. Arceo-Castillo[1], A.Y. Becerra-González[1], C.F. Aguilar-Pérez[1], J.C. Segura-Correa[1], F.J. Solorio-Sánchez[1], J.C. Ku-Vera[1]
[1]University of Yucatan, Merida, Mexico. [2]National Institute for Forestry, Agriculture and Livestock Research, Durango, Mexico (andres14_94@hotmail.com)

Energy efficiency of different physiological functions in ruminants has been a subject of interest for years. Reducing the energy requirement for maintenance in cattle might have major economic implications. It is known that thyroid hormones influence the regulation of energy expenditure in mammals. Mimosine, a non-protein amino acid present in foliage of *Leucaena leucocephala*, has been shown to induce a goitrogenic effect by affecting the synthesis of thyroid hormones. Therefore, the main objective of this work was to evaluate the effect of mimosine intake on thyroid hormones and energy metabolism of cattle. Six non-castrated Brangus bulls with an average body weight of 311 ± 17 kg and 20 ± 2 months of age were randomly allotted in a crossover design with 3 periods and 3 treatments (2 animals per treatment). Each experimental period lasted 34 d, giving 14 d of adaptation to diets and 20 d for measurement of response variables. Treatments were: control (CTL), without *Leucaena*; *Leucaena* low, (L1, 30% DMI); *Leucaena* high (L2, 60% DMI). Ratio of forage to concentrate was different between treatments: 50:50; 60:40 and 75:25 respectively. The experimental diets were formulated to supply the maintenance level of feeding. Total tract DM digestibility and urine collection were carried out in metabolic cages before cattle entered the respiration chambers for measurement of heat production (HP) and fasting heat production (FHP). Gross energy intake (GEI), digestible energy, metabolizable energy, HP and retained energy were measured in fed cattle. FHP was measured on the third and fourth day of fasting. On day 23 of each period, concentration of thyroid hormones, thyroxine (T_4) and triiodothyronine (T_3) in serum was determined. Oxygen consumption, methane and carbon dioxide production were measured while cattle were housed in two open-circuit respiration chambers. Mimosine intake was as mentioned: CTL (0 g/kg DMI), L1 (4.02 g/kg DMI) and L2 (8.04 g/kg DMI). However, no effect on T_3 and T_4 concentrations was observed among treatments ($P = 0.72$, $P = 0.57$, respectively). Similarly, there were no differences in both HP and FHP ($P = 0.36$, $P = 0.32$, respectively). In contrast, slight differences were observed in GEI and energy loss in feces ($P < 0.0001$, $P = 0.0005$). Results obtained suggest that there is no effect of mimosine on the release of thyroid hormones and therefore on HP in a maintenance diet with a low DMI. However, incorporation of *Leucaena*, a legume widely used in tropical cattle production systems, in the diet improves GEI. This work resulted in a net energy requirement for maintenance of Brangus bulls of 408 ± 50 kJ/kg$^{0.75}$/d ($n = 18$). More research is needed to elucidate the mechanisms and levels at which mimosine may affect thyroid hormones and HP in cattle.

Funding

We thank CONACYT- Mexico for financial support to the project A1-S-23910 (Efecto del alcaloide mimosa y su derivado 3,4-DHP sobre el metabolismo basal y el perfil tiroideo y metabólico de novillas).

Substitution of DDGS with canola meal in cattle finishing diets affects amino acid intake but does not affect post-ruminal amino acid flow and digestibility

S.R. Hanson, W.C. Rusche, Z.K. Smith, A.C. Menezes
South Dakota State University, Brookings, USA (AnaClara.BaiaoMenezes@sdstate.edu)

Objectives of this study were to evaluate the effects of substituting DDGS with canola meal (CM) on post-ruminal flow and digestibility of AA. Jersey steers (n = 6; BW = 257 ± 16.5 kg) with ruminal and duodenal cannulas were used in a duplicate 3 × 3 Latin square design. Diets consisted of dry-rolled corn (65%), hay (10%), liquid supplement (5%), and test ingredients (20%). Treatments were (DM basis): 1) 20% DDGS inclusion (CON), 2) 50% DDGS replacement with CM (CM50), and 3) 100% DDGS replacement with CM (CM100). Each of the three periods in the Latin Square lasted 17-d, consisting of a 10-d diet adaptation followed by a 7-d collection period. Steers were dosed intraruminally with chromic oxide (16 g/d) as an indigestible marker from d 8 to d 15. Total fecal collections were conducted from d 11 to d 15 of each period. Duodenal collections were taken from d 13 to d 15, totaling 8 samples per site in 9-h intervals, representing 24-h sampling. The post-ruminal apparent digestibility of total AA (individual, essential EAA [EAA], and nonessential AA [NEAA]) was calculated by difference between AA flow in duodenum and fecal excretion of AA, divided by duodenal flow. Ruminal utilization of individual AA (AA utilization index) was calculated as the ratio of duodenal AA flow on AA intake. A ratio greater than 1.0 indicates a net gain and a ratio lower than 1.0 indicates a net loss of the AA following ruminal degradation and microbial synthesis. Data were analyzed using the MIXED procedure of SAS, with treatment included as a fixed effect and period and animal as random effects. Linear and quadratic contrasts were applied to assess the response patterns to varying levels of DDGS and CM. Intakes of lysine, tryptophan, and glycine ($P \leq 0.047$) linearly increased with CM inclusion. Tyrosine intake was greater for CON and CM100 compared to CM50 ($P \leq 0.054$, quadratic). Despite that, the amount of EAA and NEAA reaching the small intestine, as well as the post-ruminal digestibility of AA, were not affected by treatment ($P \geq 0.20$). This response can be explained by the AA utilization in the rumen, which was similar between treatments ($P \geq 0.13$). We observed a gain of EAA in the rumen, with net gains varying from 1% for histidine to 128% for lysine. For NEAA, cysteine and proline presented a net loss in the rumen, otherwise gains varied from 3% for glutamate to 89% for glycine. This research indicates that both protein sources deliver similar metabolizable protein in finishing diets. This allows producers to prioritize cost-effectiveness and regional availability when selecting a protein source.

Characterization of packaging remnants and chemical contaminants in former foodstuff products for animal nutrition

P. Lin[1,2], M. Tretola[1], M. Zennegg[3], M. Raemy[4], S. Peiry[4], L. Pinotti[2], S. Lerch[5]
[1]Swine Research Group, Agroscope, Posieux, Switzerland. [2]Department of Veterinary Medicine and Animal Science, University of Milan, Lodi, Italy. [3]Empa, Analytical Center, Dübendorf, Switzerland. [4]Feed Chemistry Research Group, Agroscope, Posieux, Switzerland. [5]Ruminant Nutrition and Emissions, Agroscope, Posieux, Switzerland (sylvain.lerch@agroscope.admin.ch)

Former foodstuff products (FFPs) are increasingly recognized as promising circular feed ingredients that contribute to sustainable and resilient livestock production. By valorizing food industry leftovers, FFPs help reduce food waste, improve resource efficiency, and enhance feed self-sufficiency. However, their sustainable use depends not only on their nutritional benefits but also on ensuring their safety, particularly regarding potential contamination from packaging remnants and chemical contaminants. This study aimed to (1) quantify packaging residues and feed-regulated contaminants in FFPs, and (2) explore correlations among packaging materials and contaminants. Fifteen FFP samples were analyzed using Fourier-transform infrared spectroscopy (µ-FTIR) to identify packaging fragments (aluminium, plastic, and polysaccharides such as paperboard), as described in Mazzoleni et al., (2024). Standard analytical methods were used to quantify metallic trace elements (MTEs: Al, As, Cd, Pb) by inductively coupled plasma mass spectrometry (ICP-MS), while persistent organic pollutants (POPs) including polychlorinated biphenyls (PCBs), dibenzo-p-dioxins and dibenzofurans (PCDD/Fs) were analyzed by gas chromatography high resolution or tandem mass spectrometry, respectively, as described in Driesen *et al.*, (2022). Descriptive statistics for each parameter were adressed, as well as Spearman's correlation between contents in packaging fragments and each chemical contaminants using the procedure CORR of the SAS software (9.4). Packaging remnants were frequently detected, with plastic and polysaccharide residues being the most abundant (mean ± standard deviation: 6.5 ± 6.6 and 16.9 ± 11.7 pieces per 60 g of sample, respectively). MTE and POP concentrations showed substantial variability across FFP samples, but all remained below EU regulatory maximum levels and action threesholds for feed. As concentration ranged from 0.01-0.12 mg.kg^{-1} feed at 12% moisture, Pb from 0.03-0.19 mg.kg^{-1}, wheras Cd was detected in only 5 FFPs (0.05-0.06 mg.kg^{-1}). The sum of indicator PCBs ranged from 0.14-0.63 µg.kg^{-1} feed at 12% moisture and the sum toxic-equivalent (TEQ) of PCDD/Fs and dioxin-like PCBs from 0.03-0.11 ng TEQ.kg^{-1} (mid bound). Spearman's correlation revealed significant positive associations between aluminium packaging and hepta- and octa-chlorinated PCDD/F congeners ($+0.56 \le r \le +0.71$, $P \le 0.03$) as well as polysaccharide residues with the PCDD/F sum TEQ (+0.77, $P = 0.001$) and Pb (+0.59, $P = 0.03$), while plastic residues were also positively associated with Pb (+0.61, $P = 0.02$). While the detected levels of legacy contaminants do not raise immediate safety concerns, the presence of packaging remnants underscores the need for improved processing strategies to mitigate emerging physical and chemical risks. Future research should focus on refining detection methodologies and addressing safety challenges to fully harness the potential of FFPs as novel feed ingredients for resilient and sustainable livestock production.

Nitrogen degradation in different forage fiber fractions

M.B. Gomes, L.L.J.E. Jeudy, M.R. Weisbjerg, N.P. Hansen
Department of Animal and Veterinary Sciences, Research Centre Foulum, Aarhus University, 8830 Tjele, Denmark (marbg@anivet.au.dk)

Protein concentration and rumen degradation are essential for the value of the protein for ruminants. This study aimed to compare the degradation of Neutral Detergent Fiber (NDF), Acid Detergent Fiber (ADF) and hemicellulose (=NDF-ADF) with their associated nitrogen fractions, Neutral Detergent Insoluble Nitrogen (NDIN), Acid Detergent Insoluble Nitrogen (ADIN), and hemicellulose bound nitrogen (hemicellulose-N) in two varieties of each of four forage species: *Lolium perenne* (perennial ryegrass), *Festulolium* (both fescue and lolium types), and *Medicago sativa* (lucerne). Samples were harvested at Research Centre Foulum, AU Viborg in September 2024 after 43 days of regrowth with 7cm stubble, dried at 60°C for 48h, and ground to 1.5mm. Samples were incubated in two rumen-cannulated cows, in polyester bags (pore size 38µm). Incubation times were 0h (to correct the results for particle loss during washing), 8h, and 24h. At each timepoint, one bag per sample from each variety was incubated in each cow. Residues were analyzed for NDF and ADF, whereby microbial contamination was removed, and followed by nitrogen determination (Dumas method) to quantify NDIN and ADIN. Hemicellulose and hemicellulose-N contents were calculated by subtracting ADF from NDF and ADIN from NDIN, respectively. A paired t-test compared the degradability of fiber fractions and their corresponding bound nitrogen at each time point for each species. Average concentrations (% of DM) of NDF, hemicellulose, and ADF at 0h were (average±SE) 48.8±0.92, 25.4±0.78, and 23.4±0.39 for grasses, and 26.3±0.73, 9.5±0.53, and 16.9±0.74 for lucerne, respectively. NDIN, hemicellulose-N, and ADIN values (average % of DM±SE) were 1.50±0.04, 1.30±0.04, and 0.20±0.01 for grasses, and 0.76±0.01, 0.50±0.03, and 0.26±0.02 for lucerne. For all species, degradation of NDIN was higher than of NDF with an average difference of 39.2% at 8h ($P<0.003$), and 12.6% at 24h ($P\leq 0.01$). Hemicellulose-N degradation surpassed hemicellulose at 8h ($P<0.02$) by 35.4% and at 24h ($P\leq 0.03$) by 13.0%, except for *Festulolium* (perennial type) at 24h ($P=0.10$). No significant differences were found between ADF and ADIN degradation. These results suggest that hemicellulose-N degrades faster than hemicellulose, potentially due to weaker bonds between hemicellulose and N-compounds or due to the role of nitrogen as an initial binding site for microbial degradation. This pattern was not observed between ADF and ADIN, likely because lignin, a complex and largely indigestible structure is strongly covalently bonded to cell wall components and may also physically hinder microorganism access to nitrogen. Despite the hemicellulose fraction being similar or smaller than the ADF fraction, nitrogen bound to hemicellulose was four times higher than that associated with ADF, and the degradation at 24h was 24% higher. Therefore, rumen degradability of fiber-bound protein is mainly influenced by degradation of hemicellulose bound N.

Funding

The project was funded by The Danish Agricultural Agency (Copenhagen, Denmark; GUDP grant no. 34009-23-2148).

Metaproteomic and metabolomic analysis of the microbiome in pigs fed diets containing different pea varieties

F. Quinger, N. Klein, A. Camarinha-Silva, M. Rodehutscord, J. Seifert
University of Hohenheim, Institute of Animal Science, Stuttgart-Hohenheim, Germany (florian.quinger@uni-hohenheim.de)

Parts of starch and fibre in peas are resistant to enzymatic digestion in the foregut of pigs (Tan et al., 2021). The differences in resistant nutrient fractions and their implications for the hindgut microbiome in pigs across different pea varieties have not yet been studied. This study aims to identify the effect of feeding diets containing different pea varieties on the ileal and faecal microbiome of pigs using metaproteomics and metabolomics. We formulated four diets based on wheat (20%), barley (15%), soybean meal (10%), and rapeseed meal (10%) with 40% pea seeds of either a spring field pea (diet1), one of two winter field peas (diet2 and diet3), or a spring forage pea (diet4). Titanium dioxide was included as an indigestible marker. Eight barrows (25.7 ± 0.9 kg initial body weight) were fitted with a T-cannula at the distal ileum and assigned to the four dietary treatments in a 4×4 double Latin-square design. Daily feed allowance was 4% of the mean body weight. The experiment included 4 experimental periods of 11 days each with 7 days of adaption to the diet, followed by 2 days of faeces collection and 2 days of ileal digesta collection for digestibility calculation. Samples of ileal digesta and faeces were collected on the last day of each period for microbiome analyses. For protein extraction, bacterial cells were lysed by ultrasonication, and in-gel digestion was performed with trypsin. Metaproteomic analysis of peptides was conducted using an Exploris 480 mass spectrometer with an Ultimate 3000 RSLCnano system (Thermo Fisher Scientific, Darmstadt, Germany). Identification and quantification of protein groups was performed using MetaLab MAG (Cheng et al., 2022). Metabolomics was carried out using ^{1}H-NMR with a 600 MHz Avance IIIHD spectrometer (Bruker Corporation, Billerica, MA, US). Statistical analysis was conducted in R. Hindgut disappearance of total dietary fibre (TDF) and total tract digestibility of dry matter, TDF, and gross energy were lower for diet4 ($P < 0.05$). Metaproteomic analysis identified ~24,500 protein groups of which ~15,500 were retained for analysis. Most differentially abundant microbial protein groups were detected between diet4 and the other diets in ileum and faeces ($P < 0.05$). Enrichment analysis of differentially abundant protein groups revealed a lower relative abundance of ribosomal proteins in faeces for diet4. Metabolomics identified 24 and 23 metabolites in ileal digesta and faeces, respectively. Lower acetate concentrations were observed for diet4 compared to diet1 ($P < 0.05$). The results demonstrate a reduced hindgut fermentation of fibre, accompanied by decreased production of SCFA in pigs fed the diet containing the spring forage pea variety. This reduction in hindgut fermentation may be linked to reduced microbial growth, as indicated by the reduced relative abundance of ribosomal proteins.

References

Cheng, K., Ning, Z., Li, L., Zhang, X., Serrana, J.M., Mayne, J., Figeys, D., 2022. Journal of Proteome Research 22, 387-398. https://doi.org/10.1021/acs.jproteome.2c00554
Tan, F.P.Y., Wang, L.F., Gao, J., Beltranena, E., Vasanthan, T., Zijlstra, R.T., 2021. Journal of Animal Science 99, skab306. https://doi.org/10.1093/jas/skab306

Funding

Funded by the BMEL in the 'Eiweißpflanzenstrategie' based on a decision of the German parliament. Norddeutsche Pflanzenzucht Hans-Georg Lembke KG kindly provided the pea material.

Evaluating the effect of a novel feed supplement on performance, nutrient digestibility, and intestinal microbiome in pigs

J. Zentek[1], W. Vahjen[1], C. Vanderhaege[2], D. Carrion[2], M. Perpoil[2], G. Mantovani[2], K. Männer[1]
[1]Freie Universität Berlin, Berlin, Germany. [2]Cargill, Rotterdam, Netherlands (juergen.zentek@fu-berlin.de)

Nutritional strategies are key to optimizing growth, feed efficiency, and gut health in pig production. This study investigated the effects of a dietary supplement (combination of fermentation products with proteolytic acitivity and phytogenics) on growth performance, nutrient digestibility, short-chain fatty acid (SCFA) concentrations, and microbiome composition in fattening pigs. The hypothesis was, that the blend improves pig performance and digestion by modulating gut function. A total of 56 female pigs (BHZP breed) were assigned to two dietary treatments in a 35-day feeding trial: a control group receiving a basal diet and a treatment group supplemented with the test product at 1 g/kg feed. Key parameters measured included body weight gain, feed intake, feed conversion ratio (FCR), fecal consistency, ileal and total-tract digestibility, SCFA profiles, and microbial composition in the gut. The supplemented group showed significant improvements in growth performance, with a 5.3% increase in body weight gain and a 2.9% reduction in FCR compared to the control. Feed intake was slightly higher (+2.3%), supporting enhanced efficiency. Apparent ileal digestibility of crude fat (+2.9%) and nitrogen (+4.2%) was significantly improved, with trends toward increased gross energy (+3.7%) and calcium (+3.9%) digestibility. Total-tract calcium digestibility improved significantly (+4.9%), while other nutrients showed minor increases. SCFA analysis revealed a 54.6% reduction in total ileal SCFAs, particularly acetic acid (-45.0%), propionic acid (-92.2%), and butyric acid (-82.1%), suggesting reduced microbial fermentation in the small intestine, which may enhance nutrient absorption. In the colon, SCFA levels were slightly lower but not statistically significant. Colonic microbiota analysis via 16S rRNA sequencing and qPCR showed no major changes in microbial diversity between groups. Firmicutes remained dominant (~87%), while Bacteroidetes accounted for ~6%, with no major shifts. At the genus level, most dominant bacteria, including *Streptococcus*, *Lactobacillus*, and *Clostridium sensu stricto* 1, remained stable. However, *Ruminococcaceae* UCG-005 tended to be lower ($p = 0.089$), possibly reducing fiber degradation, while *Lachnospiraceae* NK4A136 group decreased significantly ($p = 0.030$). *Solobacterium* showed a slight increase ($p = 0.028$), though these changes were limited to low-abundance taxa. qPCR analysis confirmed no significant differences in total *Escherichia* spp. levels, though the heat-stable enterotoxin gene (estII) was significantly increased ($p = 0.050$) in the supplemented group, likely due to a higher plasmid load rather than pathogenic expansion. Overall, dietary supplementation improved growth performance, feed efficiency, and nutrient digestibility without major shifts in colonic microbiota. While minor taxonomic changes were observed, they were functionally irrelevant. The increase in *E. coli* estII gene copies had no negative impact on animal health, diarrhoea was not observed and the faecal scores improved form d 15-38 of treatment. These findings underscore the potential of targeted nutrition, though the underlying mode of action requires further investigation.

Funding

Cargill Premix & Nutrition, Veerlaan 17-23, 3072 AN Rotterdam, The Netherlands

Performance and nutrient digestibility in weaned piglets fed mannanase-supplemented feed

J. Schulze Holthausen[1], K. Männer[1], L. Ebersbach[1], A. Fickler[2], P. Ader[2], J. Zentek[1]
[1]Freie Universität Berlin, Berlin, Germany. [2]BASF, Lampertheim, Germany (juergen.zentek@fu-berlin.de)

Mannans, a class of non-starch polysaccharides, are commonly found in plant-based protein sources such as soybean meal (SBM), rapeseed meal (RSM), and sunflower meal (SFM) and possess anti-nutritional characteristics. Monogastric animals, including pigs, lack the endogenous enzymes required to degrade these complex carbohydrates, leading to reduced nutrient digestibility and potential negative effects on gut health. Mannanase enzymes, such as endo-1,4-β-D-mannanase, can break down mannans, thereby mitigating their anti-nutritional effects and potentially enhancing nutrient utilzation and overall animal performance. This study aimed to assess the impact of an endo-1,4-β-D-mannanase (Natupulse® TS, BASF SE, Germany) on growth performance, nutrient digestibility, and intestinal function in weaned piglets. A total of 32 weaned piglets were housed in pairs and assigned to one of two dietary treatments over a 21-day trial following a four-day adaptation period. The control group received a diet composed of corn, SBM, RSM, and SFM with reduced nutrient density, while the test group received the same diet supplemented with endo-1,4-β-D-mannanase (800 TMU/kg). Growth performance indicators, including body weight gain, feed intake, and feed-to-gain ratio, were recorded weekly. Additionally, apparent praecaecal digestibility of crude protein, amino acids, crude fat, calcium (Ca), and phosphorus (P) was evaluated through ileal digesta sampling on days 21-24. Electrophysiological analyses were conducted on jejunal tissue samples using Ussing chambers to assess glucose-stimulated electrogenic transport. Statistical analyses were performed using univariate ANOVA, with significance set at $P \leq 0.05$. Piglets supplemented with mannanase exhibited a higher body weight gain (8.72 ± 0.39 kg) compared to the control group (8.15 ± 0.71 kg, $P = 0.069$). Feed efficiency was significantly improved, with the feed-to-gain ratio decreasing from 1.73 in the control group to 1.62 in the supplemented group ($P = 0.003$). The inclusion of mannanase led to significant improvements in apparent praecaecal digestibility of crude protein (from 69.2% to 73.8%, $P = 0.003$) and crude fat (from 79.9% to 83.2%, $P = 0.041$). Additionally, digestibility of Ca (39.0% to 41.8%, $P = 0.045$) and P (38.0% to 42.2%, $P = 0.026$) was enhanced. Significant increases were also observed in the digestibility of amino acids, including valine ($P < 0.001$), threonine ($P = 0.028$), and methionine ($P = 0.031$). Moreover, piglets receiving mannanase exhibited an improved glucose-stimulated electrogenic transport response ($P = 0.044$). Supplementing piglet diets with endo-1,4-β-D-mannanase significantly enhanced feed efficiency and nutrient digestibility. These findings suggest that incorporating this enzyme into piglet feed containing mannan-rich ingredients could be beneficial for improving growth performance and nutrient utilization.

Funding
This study was funded by BASF SE.

Lanthanide citrate improves feed conversion ratio and enhances growth performance of weaned piglets

S.B.E. Rongen, I.C. Belt, van den, I.M. van As, L.C.M. van Enckevort
Denkavit, Voorthuizen, Netherlands (s.rongen@denkavit.com)

The post-weaning period is a stressful period for the piglet, which can have negative effects on feed intake and increase the risk of colonization by pathogens. To overcome this problem, zinc oxide (ZnO) and antibiotics have been used as solutions for many years. However, the European Union banned the use of increased pharmaceutical dosages of ZnO in June 2022. Since then there is an ongoing search for sustainable nutritional interventions to support gut health of piglets during and after weaning. One of these interventions could be the addition of lanthanide citrate (Terragut) to the diet, an EFSA approved feed additive. A trial was conducted at Denkavit Innovation Centre (Voorthuizen, The Netherlands). The aim of this trial was to study the effects of lanthanide citrate and ZnO supplementation in a standard performance weaner diet on the growth performance and intestinal health of weaned piglets. 420 Topigs TN70 x DanBred Duroc piglets were weaned at 28 days of age and allocated to one of the three dietary treatments: (1) a standard performance weaner diet, (2) the standard diet supplemented with 2500 ppm ZnO and (3) the standard diet supplemented with 250 ppm lanthanide citrate. Experimental diets were provided for 14 days post-weaning after which all piglets received a standard performance rearing diet. Body weight and feed intake were recorded per pen on days 8, 15 and 36, with individual body weights measured at weaning. Faecal colour and consistency were assessed on days 5, 8, 12, 15, 19, 22, 29 and 36 using a five-point scoring system. Data was analysed with an ANCOVA model with weaning weight as covariate. Results show that inclusion of lanthanide citrate significantly improves average daily weight gain during the weaning period from day 1-15 compared to ZnO (282 vs. 245 vs. 300 g/piglet; $P<0.001$), which is partly attributable to the second week of weaning from days 8-15 (395 vs. 331 vs. 417g/piglet; $P<0.001$). Furthermore, the inclusion of lanthanide citrate significantly improves FCR during the weaning period from day 1-15 compared to ZnO (1.16 vs. 1.26 vs. 1.11; $P<0.001$). Feed intake was similar between treatment groups. No significant differences in performance were found after the transition to the standard rearing diet from day 15-36. Faecal scores for colour and consistency were improved for both the ZnO and lanthanide citrate treatments compared to the standard diet treatment. This suggests a positive effect of lanthanide citrate on intestinal health. This study showed that the inclusion of lanthanide citrate to a weaner diet resulted in a significantly higher weight gain and improved FCR of piglets during the weaning period. The results of this study provide insight into the impact of dietary interventions on post-weaning performance and gastrointestinal health in piglets.

The use of carbohydrases in growing pig diets with brewers' spent grain increases energy and nutrient digestibility without affecting growth performance

L. Piquer[1], O. Piquer[2], P. García-Rebollar[3], E. Pérez-Calvo[4], A. Calderón[5], D. Belloumi[1], A. Cerisuelo[1]
[1]Centro de Investigación y Tecnología Animal, Instituto Valenciano de Investigaciones Agrarias, Segorbe, Spain. [2]Departamento de Producción y Sanidad Animal, Salud Pública Veterinaria y Ciencia y Tecnología de los Alimentos, Universidad CEU-Cardenal Herrera, Valencia, Spain. [3]Departamento de Producción Agraria, ETSIAAB, Universidad Politécnica de Madrid, Madrid, Spain. [4]dsm-firmenich, Kaiseraugst, Switzerland. [5]dsm-firmenich, Madrid, Spain (piquer_lai@gva.es)

The use of agroindustrial by-products, such as brewers' spent grain (BSG), to replace conventional energy and protein sources in pig diets can reduce the environmental impact and food-feed competition in pig production. However, BSG's high content in non-starch polysaccharides (NSP), mainly composed of arabinoxylans, can decrease nutrient digestibility. Several studies have shown that using adequate exogenous enzymes can improve nutrient digestibility of fibrous diets in young animals. However, the effect on growing pigs is less evident (Torres-Pitarch et al., 2019; Petry et al., 2020). An experiment was conducted to evaluate the effect of adding carbohydrases in growing pigs' diets with BSG on performance and nutrient digestibility. To this aim, 120 pigs of 75.7 ± 5.93 kg body weight (BW) were distributed in three isonutrient experimental diets (8 pens/diet): a control diet, a diet with 20% BSG and a diet with 20% BSG supplemented with an endo-1,4-beta-xylanase at 112 FXU/kg feed. Average daily weight gain (ADG) and feed intake (ADFI) were measured over the experiment, and feed conversion ratio (FCR) was calculated. Faecal samples were obtained from the rectum of two animals per pen for 3 consecutive days, pooled, and processed to determine the energy and nutrient coefficient of total tract apparent digestibility (CTTAD), using acid insoluble ash as a marker in feeds. The inclusion of BSG (with or without enzyme supplementation) did not affect the ADG (1.08 kg/d on average; $p=0.740$), ADFI (2.81 kg/d on average; $p=0.653$), FCR (2.59 kg/kg on average; $p=0.880$) and final BW (103 kg on average; $p=0.990$). The inclusion of carbohydrases in the diet with BSG led to a higher ($p<0.05$) CTTAD of energy (GE), protein (CP), neutral detergent fiber (NDF) and acid detergent fiber (ADF) fractions compared to the diet without enzyme (0.832 vs. 0.798, 0.809 vs. 0.782, 0.626 vs. 0.475 and 0.436 vs. 0.343 for CTTAD of GE, CP, NDF and ADF, respectively). The control group showed similar CTTAD of GE and CP and lower ($P<0.05$) CTTAD of the fiber fractions compared with those of the group fed with BSG and enzymes. The CTTAD of fat was higher in both diets with BSG compared with control (0.755 vs. 0.539; $p<0.05$). In conclusion, supplementing diets with BSG with carbohydrases improved dietary energy and nutrient utilization, especially for protein and fiber fractions, without affecting pig performance.

References

Petry, A.L., Patience, J.F., 2020. Journal of Animal Science 98, skaa318. https://doi.org/10.1093/jas/skaa318

Torres-Pitarch, A., Manzanilla, E.G., Gardiner, G.E., O'Doherty, J.V., Lawlor, P.G., 2019. Animal Feed Science and Technology 251, 153-165. https://doi.org/10.1016/j.anifeedsci.2018.12.007

Funding

Spanish Ministry of Science and Innovation (RTI-2018-095246-B-C21 and C22) and Generalitat Valenciana (IVIA-GVA 52201L project, cofinanced by the EU through the ERDF Program 2021-2027 CV). Thanks to Vall Companys Group for providing the animals and technical support and to PORTESA for their support on feed formulation and manufacturing.

Increasing levels of phytase supplementation in diets for nursery pigs increases the digestibility of amino acids

A.M. Veira[1], J.P.R. Gonçalves[1], C.A. Silva[1], I. França[1], M.T. Ortiz[1], L.D. Campos[1], D.A. Marçal[1], A.Z. Fraga[1], C.C.R. Silva[1], G.S.C. Paulino[1], A.F. Oliveira[1], S.J. Sardinha[1], E. François[2], L. Hauschild[1]
[1]São Paulo State University (Unesp), Jaboticabal, Brazil. [2]Kemin Industries, Valinhos, Brazil (alini.mari@unesp.br)

Most of the phosphorus (P) present in plant-based ingredients is bound to phytate, making it unavailable to pigs. Therefore, the use of inorganic sources of P in feed is necessary. However, another nutritional strategy that has been employed is the supplementation of diets with phytase, an exogenous enzyme capable of breaking down the phytate, making P available for use by animals. Additionally, the use of phytase in diets may also improve the availability of other nutrients such as amino acids (AA). However, limited information is available about the impact of phytase supplementation on AA digestibility. Therefore, the objective of this study was to evaluate the ileal digestibility (ID) of AA in nursery pigs fed diets supplemented with increasing levels of phytase. A total of 36 pigs (17.5 ± 1.2 kg) were assigned to a randomized block design distributed in six treatments. A positive control (PC) diet was formulated to meet the nutritional requirements of nursery pigs. A negative control (NC) diet was formulated with a reduction of 50% in total P, 72% in available P, 44% in calcium (Ca), as well as a 36 kcal/kg reduction in metabolizable energy (ME). Four additional diets that were similar to the NC, but contained 250, 500, 1,000 and 2,000 FTU of a commercial phytase per kg of diet were also used. Pigs were housed individually in metabolic cages for 12 days and on day 13 were anesthetized and euthanized. Ileal digesta was collected from the final third of the small intestine, lyophilized and subjected to the analysis of lysine (Lys), leucine (Leu), threonine (Thr), methionine (Met), cysteine (Cys), tryptophan (Trp), valine (Val), and isoleucine (Ile) composition. Data were analyzed using a randomized block design. Contrasts were constructed to compare PC vs NC, as well as to test the effect of phytase using NC vs levels of 250, 500, 1,000, and 2,000 FTU/kg. The effect of increasing phytase levels from 0 (NC) to 2,000 FTU/kg (excluding PC) was evaluated using linear and quadratic regression analysis. Polynomial orthogonal contrast analysis was used to compare PC vs levels of 250, 500, 1,000, and 2,000 FTU/kg. Differences were considered significant at $P < 0.05$. The ID of the evaluated AA was higher ($P < 0.05$) for PC when compared to NC (82 vs 76%). When comparing PC with treatments with phytase supplementation, there was no effect ($P > 0.05$) on the ID of the AA (82 vs 80%). However, a quadratic increase ($P < 0.05$) was observed in the ID of the AA as the inclusion of phytase increased in the diets. Therefore, phytase supplementation in diets without inorganic phosphorus source improved ID of AA for pigs in the nursery phase.

Funding

This research was supported by Kemin Industries. Alini Mari Veira was supported by the National Council for Scientific and Technological Development – CNPq (grant number: 151531/2024-2).

The impact of microbial-derived metabolites on gut function in weaned piglets under different levels of dietary crude protein and a surplus of dietary amino acids

L.H. Chen[1], M.S. Hedemann[1], H. Pfannkuche[2], S. Purup[1], N. Canibe[1]
[1]Aarhus University, Tjele, Denmark. [2]Universität Leipzig, Leipzig, Germany (lihsuan.chen@anivet.au.dk)

High dietary protein (DP) can promote growth of weaned piglets but increases the risk of post-weaning diarrhea (PWD) possibly by altering microbiota composition and leading to high concentration of metabolites from microbial protein fermentation. Sloth et al. (2022) found that a high-DP diet supplemented with 35% extra crystalline lysine-HCl above the ideal protein profile [2] and with corresponding increases in methionine (32%), threonine (62%), tryptophan (22%), and valine (67%) reduced diarrhea treatment days by 50%. This study examined the effects of DP level and excessive crystalline amino acid (EAA) supplementation to a high-DP diet, similar to that in (1), on piglet gut health, focusing on gut metabolite concentration and epithelial cell viability. A total of 120 piglets were assigned to three dietary treatments: low-protein (15% DP, LP), high-protein (25% DP, HP), and high-protein with extra EAA (25% DP + EAA according to [1], HPEAA). The study was conducted in five blocks, each with 24 piglets allocated to six pens (four per pen; two pens per treatment). One piglet per pen was slaughtered on days 7 and 28 post-weaning (PW) for digesta collection from distal small intestine and colon. Freeze-dried digesta was analyzed for untargeted metabolomics using the LCMS-9030 Q-TOF MS system. Cell-based assays using porcine jejunal (IPEC-J2) and colonic (PoCo83-3) cell lines evaluated the effect of digesta from the three treatment groups and selected protein fermentation-derived compounds on cell viability. HP and HPEAA diets altered the metabolite profile, especially in the hindgut, compared to LP. Cyclic phosphates were higher in the HP and HPEAA groups than in the LP group on day 7 PW (Variable importance in projection scores (VIP) > 2). By day 28 PW, metabolites from purine, fatty acid, and bile acid metabolism differed between the LP and HP/HPEAA groups ($p<0.05$). AMP was higher in digesta from HPEAA piglets than HP piglets ($p<0.05$) and tyramine increased in HP group compared to LP ($p<0.05$). In the PoCo83-3 cells, colonic digesta from HP and HPEAA piglets reduced epithelial cell viability at a lower concentration than LP, indicating a more harmful impact of the two high protein groups. Protein fermentation metabolites (p-cresol, tyramine, and NaHS) reduced cell viability at concentrations equivalent to those measured in the HP group *in vivo*. However, purine metabolites (AMP and inosine) supported cell growth under specific p-cresol-induced harmful conditions. Increasing DP shifted hindgut metabolite profiles on days 7 and 28 PW, with HP and HPEAA groups showing similar patterns and distinct from the LP group. Cell viability results aligned, indicating a milder effect of digesta from LP pigs than that of HP or HPEAA pigs, and metabolite interactions influenced cell viability. The reported differences in diarrhea between HP and HPEAA were not explained by metabolite profiles.

References

Sloth, N.M., Krustrup, A.K., Grove, S.S., Rønvig, E., Tybirk, P., Bache, J.K., Willkan, M., 2022. MEDDELSE Nr. 1263 – Fire protein- og fem aminosyre niveauer i foder til smågrise. SEGES INNOVATION. https://www.landbrugsinfo.dk/-/media/landbrugsinfo/public/1/e/b/fire_protein_og_fem_aminosyreniveauer_i_foder_til_smagrise_1263.pdf
Tybirk, P., Sloth, N.M., Bruun, T.S., Hales, J., 2024. Danish nutrient standards. https://pigresearchcentre.dk/Nutrient-standards

Funding

This study was conducted as part of the PIG-PARADIGM project, funded by the Novo Nordisk Foundation (Grant No. NNFSA210073688).

Effect of protein level and essential amino acid supplementation on indicators of gut health in weaned piglets

M.H. Kroier[1], H.N. Lærke[1], N.M. Sloth[2], A.A. Schönherz[1], N. Canibe[1]
[1]Department of Animal and Veterinary Sciences, Aarhus University, Foulum, Denmark. [2]SEGES innovation P/S, Aarhus, Denmark (mhk@anivet.au.dk)

Supplementing with extra essential amino acids (EAA) may reduce the occurrence of post-weaning diarrhoea (PWD) associated with high-protein diets. Sloth et al. (2022) found a reduction in PWD treatment days by more than 50% when supplementing 35% extra crystalline lysine-HCl above the ideal protein profile with corresponding increases in methionine, threonine, tryptophan and valine by 32%, 62%, 22% and, 67%, respectively (Sloth et al., 2022). In the current study, 120 weaners (~28d old) housed four piglets per pen, were fed one of three diets: Lp (15% crude protein (CP)), Hp (25% CP), and HpEAA (Hp with EAA supplementation according to (Sloth et al., 2022)) for four weeks. Individual body weight and feed intake (FI) per pen were registered weekly. Faeces from each pen were scored daily for diarrhoea. On d7 and 28 post-weaning (PW), 10 piglets per dietary group were euthanized to sample digesta from stomach (STO), distal small intestine (SI3), cecum (CAE), proximal (CO1), mid (CO2), and distal (CO3) colon for bacterial quantification and microbial metabolite analyses. Linear mixed-effects models were used to assess statistical differences. From week 2, Lp piglets (9-17%) had a lower probability of diarrhoea than Hp (65-91%) and HpEAA (43-62%) ($P \leq 0.02$), and from week 3, HpEAA (43-50%) had lower probability than Hp (65-71%) ($P \leq 0.03$). On d28 PW, Hp and HpEAA piglets had higher branched-chain fatty acid concentrations in CO2 and CO3 ($P \leq 0.04$) compared to Lp. Further, both Hp groups showed higher hydrogen sulphide concentration in the CAE ($P \leq 0.01$), while Hp piglets had a higher concentration than Lp in CO2 ($P=0.01$), with HpEAA being intermediate. Additionally, lactic acid concentration was higher in STO and SI3 ($P \leq 0.009$) in HpEAA piglets compared to the other groups. Quantification of *E. coli* in digesta showed that Hp and HpEAA groups had higher log copies /g sample ($P \leq 0.04$) compared to Lp in CAE and CO1 on d7 PW. Hp and HpEAA also tended ($P \leq 0.09$) to have more Lactobacilli in CAE on d28. Piglets fed Hp had the highest average daily gain (0.379kg/day) overall, followed by HpEAA (0.314kg/day) and Lp (0.281kg/day), with an LSD of 0.089 kg/day. HpEAA piglets had the lowest average daily FI at 326g/day, compared to 424g/day for Hp and 427g/day for Lp. The gain-to-feed ratio was higher in both Hp groups ($P<0.01$), compared to Lp. Although, this study was not designed to conclude on production results, the effect of protein- and amino acid levels on performance parameters and diarrhoea occurrence align with those of Sloth et al. (2022). While some metabolites may provide insights into the observed difference in diarrhoea, bacterial quantification and metabolite analyses did not fully explain the underlying mechanisms, warranting further data exploration.

References

Sloth, N. M., Krustrup, A. K., Grove, S. S., Rønvig, E., Tybirk, P., Bache, J. K., & Willkan, M., 2022. MEDDELSE Nr. 1263 - Fire protein- og fem aminosyre niveauer i foder til smågrise, SEGES INNOVATION. https://www.landbrugsinfo.dk/-/media/landbrugsinfo/public/1/e/b/fire_protein_og_fem_aminosyreniveauer_i_foder_til_smagrise_1263.pdf

Funding

This study was conducted as part of the PIG-PARADIGM project, funded by the Novo Nordisk Foundation (Grant No. NNFSA210073688).

Including olive cake in pig diets revealed changes in the fecal and serum metabolomic profile

D. Belloumi[1,2], M.I. Roca[3], P. García-Rebollar[4], L. Piquer[1], A. Cerisuelo[1]
[1]Centro de Investigación y Tecnología Animal, Instituto Valenciano de Investigaciones Agrarias, Segorbe, Spain. [2]Institute for Animal Science and Technology, Universitat Politècnica de València, Valencia, Spain. [3]Unidad Analítica, Instituto de Investigación Sanitaria La Fe, Valencia, Spain. [4]Departamento de Producción Agraria, Escuela Técnica Superior de Ingeniería Agronómica, Alimentaria y de Biosistemas, Universidad Politécnica de Madrid, Madrid, Spain (cerisuelo_alb@gva.es)

The study of fecal and serum metabolites can help reveal insights into the interplay between diet and host metabolism. Olive cake (OC), a by-product from the olive oil industry, is rich in insoluble fiber, oleic acid, and phenolic compounds, which can potentially affect gut microbiota and metabolism. The effects of including two types of OC in growing pig diets on faecal and serum metabolomics were evaluated. Thirty 54.1±4.84 kg pigs were assigned to three dietary groups: a control group (C), a group fed with 200g/kg of partially defatted OC (PDOC), and a group fed with 200g/kg of cyclone OC (COC) for 21 days. Fecal and blood samples were collected at the end of the experiment for metabolite extraction and untargeted metabolome analysis. After data pre-processing and filtration, the orthogonal partial least squares discriminant analysis (OPLS-DA) was computed to determine dissimilarities among groups. Then, the selected molecular features that discriminated among treatments were annotated using the online CEU Mass Mediator and the Livestock Metabolome Database. The analysed chemical composition of OC revealed differences in the crude fat (CF), insoluble (IF) and soluble fibre (SF), being the CF and SF content higher in COC than PDOC (CF: 168 vs. 116 g/kg and SF: 75.4 vs. 55.3 g/kg) and the IF higher in PDOC compared with COC (506 vs. 392 g/kg). Regarding phenolic compounds, both OC and the diets that included them contained hydroxytyrosol, tyrosol, verbascoside, oleacein, and p-coumaric acid, whereas no one of these compounds was detected in the C diet. Regarding faecal metabolites, results revealed a clear separation between C and 20PDOC, C and 20COC, and 20PDOC and 20COC groups. Most of the tentative metabolites connected to the discriminant ($P<0.05$) molecular features that were upregulated in the feces of animals fed 20PDOC vs. those fed C or 20COC were linked to lipid metabolism. However, between 20COC and C, the most discriminant fecal molecular features that were upregulated in 20COC were polyphenolic compounds (flavans, bioflavonoids and polyflavonoids). In serum metabolites, no discriminant features were observed between 20PDOC and 20COC animals. But, the comparison between C and 20PDOC animals showed changes in glycerophospholipids, glycerophosphocholines (upregulated in 20PDOC animals). When comparing C and 20COC animals, wide variety of functional groups were upregulated in animals fed 20COC, such as amines, benzene and substituted derivatives, organooxygen compounds, heteroaromatic compounds, pyrrolizines, phenols, carboxylic acids and derivatives, among others. Esters were downregulated in both groups fed OC compared with C. The results support differences in fecal and serum metabolites among the groups that were generated by the ingestion of OC. Indeed, OC can modify the fecal and general metabolism of animals, impacting specific metabolic functions that are different depending on the type of OC.

Funding

This work was supported by the Spanish Ministry of Science and Innovation (Project RTI-2018-095246-B-C22) and Project IVIA-GVA 52201L from IVIA, co-financed by the EU through the ERDF Program 2021-2027 CV. Dhekra Belloumi is a recipient of the Santiago Grisolía PhD scholarship (GRISOLIAP/2020/023).

Effects of guanidinoacetic acid supplementation on the sow milk quality and its effects on piglet weight development during lactation

S. Jamal Esfarjani Nejad[1,2], L. Schneider[2], P. Loibl[3], J. Steinhoff-Wagner[1], G. Dusel[2]
[1]Technical University of Munich, Freising-Weihenstephan, Germany. [2]University of Applied Sciences, Bingen, Germany. [3]Alzchem Trostberg GmbH, Trostberg, Germany (g.dusel@th-bingen.de)

Large variation in birth weights within litters in highly prolific sows lead to increased suckling piglet mortality, animal suffering, reduced piglet weaning weight, and has negative effects on piglet rearing. Creatine is an essential component of cellular energy metabolism in vertebrates. It is synthesized endogenously from glycine, arginine, and methionine. Guanidinoacetic acid (GAA) is the direct precursor of creatine and is used as a creatine source in feed. The feeding GAA to gestating sows could increase the number of live born and the birth weight of piglets and to lactating sows could result heavier piglet weight and larger litters at weaning. Support the endogenous creatine synthesis, dietary GAA supplementation could lead to higher creatine concentrations in sow milk, potentially enhancing piglet growth. This study aimed to investigate whether GAA supplementation improves the creatine content in sow colostrum and milk and enhances piglet weight development during lactation. A Total of 93 Topigs TN70 sows were allocated to two feeding groups (CON, n=49, CON + 0.1% GAA, n=44) based on parity, body weight (BW), backfat thickness (BFT), body condition score (BCS), and previous litter performance. The feeding study was divided into three trial phases: gestation (days 0–108), transition (days 108–115), and lactation (days 115–143). Sows' BW, BFT and BCS were recorded at insemination (day 0), transition (day 108), and weaning (day 143). After farrowing, litter performance and piglet birth and weaning weights were recorded during lactation. Colostrum samples were collected immediately after birth of the first piglet, and milk samples were taken on day 10 of lactation. In the ANOVA (R 4.4.2), feeding group and parity were considered as fixed effects. The sows' performance parameters including BW, BFT, BCS, and the number of live and stillborn piglets at farrowing were comparable between the two feeding groups. Litter weight gain, piglet average daily gain and Brix values of colostrum and milk samples showed no differences between the two feeding groups. The creatine content in colostrum tended to be higher in the GAA group compared to the CON group (mean ± SE 194.9 ± 15.5 mg/kg *vs.* 160.9 ± 8.7 mg/kg; $P = 0.063$). The creatine content in milk was comparable between both feeding groups. Piglet birth weight was significantly higher (1.323 ± 0.01 kg *vs.* 1.270 ± 0.01 kg; $P = 0.006$) and weaned piglets tended to be heavier in the GAA group (7.554 ± 0.07 kg *vs.* 7.360 ± 0.07 kg; $P = 0.056$). In conclusion, GAA to supplementation the sows' diets led to a significantly higher birth weight and a trend towards a higher weaning weight of the piglets. This was likely due to tendentially increased creatine levels in colostrum.

References

Mendoza, S. M., Richert, S., Htoo, J. K., 2022. Archivos Latinoamericanos de Producción Animal. 2022. 30 (Supl. 1) XXXII Reunión Internacional de Producción de Carne y Leche en Climas Cálidos, October 6–7, 2022. https://www.researchgate.net/publication/364444640_Effect_of_Guanidinoacetic_acid_a_creatine_precursor_in_gestating_and_lactating_sows

Schneider, L., Schuh, K., Dusel, G., Bauer, L., Richert, S., Htoo, J. K., 2022. Effects of Adding Guanidinoacetic Acid to Sow Feed During Gestation and Lactation on Sow and Litter Performance. 15th International Symposium on Digestive Physiology of Pigs. https://www.sciencedirect.com/journal/animal-science-proceedings/vol/13/issue/2

Effects of citrusflavonoids and their respective aglycones on apparent prececal nutrient digestibility and antioxidant status of growing broilers

A.S. Mueller[1], K. Syriopoulos[1], R. Tona[1], D. Kofel[1], R. Boehlen[1], K. Maenner[2]
[1]ERBO AG, Buetzberg, Switzerland. [2]Free University Berlin, Berlin, Germany (mueller.andreas@erbo.ch)

In farm animals the dietary inclusion of citrusflavonoids (FLA) and/or their aglycons (AGL) improved growth rate, gastric acid secretion (Li et al., 2025), lipid metabolism, and liver glutathione reductase activity (Hager-Theodorides et al., 2021). To date no information exists on the influence of citrusflavonoids (FLA) or their aglycons (AGL) on nutrient digestibility. Two-hundred-and-seventy day old Cobb500 broiler chickens (41.3±0.63 g) were assigned to groups Con (no additive), FLA and AGL and placed into 18 pens. Thus, all groups included 6 repetitions with 15 birds each. Birds were fed standard diets for 42 days. Dietary levels of the flavonoids hesperidin (15.31 mg/kg diet), neohesperidin (3.56) and naringin (18.31) in group FLA represented their concentration in 60 mg/kg of a commercial citrus flavonoid blend. Dietary aglycon levels in group AGL (hesperitin 9.30 mg/kg diet and naringenin 8.57 mg/kg diet) were calculated, assuming a 1:1 cleavage of the entire flavonoids. Titanium-IV-oxide as an inert digestibility marker was included into all diets. Performance parameters were recorded at days 14 and 42. On day 42 broilers were euthanized and prececal nutrient digestibility was determined from pooled small intestinal digesta samples. Reduced and oxidized glutathione (GSH and GSSG) in plasma were determined using a commercial Kit. For data analysis a one-way ANOVA was performed. During the entire experimental period cumulative feed intake did not differ between groups. In contrast, on day 42 groups FLA (3478 ± 30.8 g) and AGL (3407 ± 30.8) showed a significantly ($p<0.001$) elevated body weight compared to Con broilers (3330 ± 27.7), resulting from improved FCR ($p<0.001$). FCR was 1.490 ± 0.011 g/g (Con), 1.431 ± 0.004 (FLA) and 1.455 ± 0.001 (AGL). Apparent prececal digestibility of dry matter, protein and ash did not differ among groups. In contrast, FLA and AGL increased apparent prececal digestibility of energy (78.8 ± 0.65 and 78.7 ± 1.44 %), and fat (92.2±0.65 and 91.5. ±0.40 %) compared to Con broilers (70.6 ± 2.86 and 88.8 ± 3.12%) significantly ($p <$ min. 0.05). Plasma GSH/GSSG ratio was significantly ($p>0.05$) increased from 1.21 ± 0.086 in group Con to 1.89 ± 1.03 and 2.74 ± 0.85 in groups FLA and AGL, respectively. It can be concluded that both, FLA, and their AGL, represent promising phytogenic molecules for the improvement of broilers' performance and health (1). Their performance enhancing effect obviously results from their influence on prececal energy- and fat digestibility. In contrast phenolic essential oils and pungents have been reported to increase primarily protein- and ash digestibility. The positive change in plasma GSH by FLA and AGL may result from increased glutathione reductase activity, as reported earlier (2). Selective effects of phytogenic molecules on energy/fat-digestibility or protein/amino acid-digestibility need further intensive investigation in the future.

References

Li, Y., Zhong, M., Wan, S., et al., 2025. Poultry Science 104, 104781.
https://doi.org/10.1016/j.psj.2025.104781
Hager-Theodorides, A., Massouras, T., Zoidis, E., et al., 2021. Foods 10, 739.
https://doi.org/10.3390/foods10040739

Effect of mannanase supplementation in diet programs on performance of broiler chickens

A. Fickler[1], E. von Heimendahl[1], A. Sokale[2], B. Turner[3]
[1]BASF SE, Ludwigshafen, Germany. [2]BASF Corporation, Florham Park, NJ, USA. [3]Poultry Research Partners, LLC, Athens, GA, USA (elke.von-heimendahl@basf.com)

Beta-mannans in feedstuffs can increase digesta viscosity, reduce digestibility and impair health and performance of monogastric animals. Beta-mannanase mitigates these effects but information on energy and nutrients made available by supplementing mannanase in addition to other enzymes is lacking. Different mannanases might differ in efficacy based on their pH optimum. The study evaluated effects of two beta-mannanases in different diet programs on broiler performance. One-day-old Cobb 500 male chickens were allocated to four treatments with 12 pens of 32 birds each. Treatments were positive control (PC) diet: 1000 FTU/kg phytase plus xylanase & glucanase. PC was reduced in energy by 45 kcal/kg and amino acids by 5% (negative control (NC)). NC was supplemented with either Mannanase-A (NC+A) or mannanase-B (NC+B) at recommended levels. Diets were corn-soybean meal based and fed ad libitum P1 (d1-14), P2 (d15-28), P3 (d29-42), P4 (d43-56). Body weight (BW), feed intake and mortality were measured day 1, 14, 28, 42, 56. Feed conversion ratio (FCR) was calculated per pen. Mannanase pH profile was evaluated using a dinitrosalicylic acid (DNS) assay. Mannanase-A shows highest activity between pH2-5.5, mannanase-B is more active at pH 6-8. In P1 no difference in BW but significantly higher FCR in NC compared to other treatments was found. At the end of P2, NC showed reduced BW compared to other treatments. FCR was significantly lower in PC and NC+A compared to NC and NC+B. At the end of P3 (42 days) highest BW was observed in mannanase-A treatment and numerically lowest in NC. For the overall trial period (d0-56), NC showed numerically lower BW compared to all other treatments. The FCR differed significantly between NC (1.75) and NC-A (1.71). PC and NC-B had a value of 1.73. 45 kcal/kg energy and 5% amino acids reduction decreased performance in NC. Mannanase addition compensated this reduction. Different compensation indicates that mannanase-A might be more efficient related to its optimum activity in acidic environment in crop, proventriculus and gizzard, allowing earlier breakdown of mannan structures compared to mannanase-B, with relevant activity starting beginning of duodenum according to pH profile. Mannanase supplementation on top of phytase, xylanase and glucanase improves performance but pH dependent mannanase activity should be considered. Specifically, mannanase-A showed promising results in enhancing performance when used in a diet reduced in energy and amino acids.

Net energy for feed additives evaluation for broilers and potential benefits of enzymes

P. Cozannet, C. Gras, T. Mahmood, A. Cayzac, Y. Mercier
Adisseo, Saint Fons, France (Pierre.Cozannet@adisseo.com)

The importance of energy in swine and poultry production is key to the economic significance of feed costs and energy's role as the most expensive component. Net Energy (NE) is the more accurate measure of energy available for animal maintenance and production compared to Digestible Energy (DE) and Metabolizable Energy (ME**)**. Enzyme (enz) additives have previously been demonstrated to improve nutrient digestibility and capacity to improve energy and protein utilization and animal performance. If ME were fully suitable for the evaluation of digestibility effect, protein utilization might only be measured by NE and heat increment measurement. Meta analysis was performed using data gathered from metabolisable and net measurement trials performed for exogenous enz evaluation. Data were analysed using the MIXED procedure of SAS (SAS, 2008) considering the experiment as a random effect. Dataset included experiment (n=8), diets (n=34) and enz level (n=2). Diet nutritional values (i.e. ME, ME for zero nitrogen balance MEn and NE) were correlated with various dietary factors, such as starch, crude protein, and fiber content. Enzyme significantly affected all parameters (ME/GE, MEn/GE, NE/ME, ME, MEn and NE; $P < 0.01$). ME and NE contents were significantly increased by 93 and 117 kcal/kg DM by enz supplementation. Improvement was 2.7 and 4.6% with enz supplementation relative to control ($P < 0.01$). Soluble and insoluble arabinoxylan (AX) were found as the most significant parameters to explain the enzyme effect on the diet. Optimal prediction of ME and NE were obtained taking into account crude protein, crude fat and NDF. Factors were significantly affected by enzyme ($P < 0.001$). R square were 0.88 and 0.92 for ME and NE, respectively. Results from the study show that enz supplementation significantly improved ME and NE content in feed, leading to enhanced energy utilization in broiler chickens. The text also discusses the impact of soluble and insoluble fiber on digestibility and energy efficiency, with enz supplementation reducing the detrimental effects of fiber. Similarly, correlation was established between substrate and enz effect. Result suggests a negative impact of soluble AX on energy metabolisability and of insoluble AX on NE to ME ratio. Enzyme alleviated the detrimental effect of both soluble and insoluble AX with 0.46 and 0.88 R square, respectively. In conclusion, understanding the mechanisms by which enz affect energy utilization in feed is important. Enzyme supplementation can potentially improve energy efficiency in poultry production. It also suggests the need to develop equations to predict enz effects for better diet reformulation.

Effect of various methods to enhance the efficiency of canola meal in pelleted calf starter mixture

P. Górka[1,2], K. Krupa[1], M. Podżorski[1], M. Przybyło[1], J. Kański[1], Z.M. Kowalski[1], A. Stępień[3], K. Burakowska[4], G.B. Penner[4], A. Rogiewicz[5], R. Patterson[6]
[1]University of Agriculture in Krakow, Kraków, Poland. [2]National Research Institute of Animal Production, Balice, Poland. [3]The Polish Association of Oil Producers, Warszawa, Poland. [4]University of Saskatchewan, Saskatoon, Canada. [5]University of Manitoba, Winnipeg, Canada. [6]CBS Bio Platforms, Calgary, Canada (pawel.gorka@urk.edu.pl)

Three studies were conducted to determine whether lysine supplementation, feed enzyme supplementation, and extrusion improve the efficiency of canola meal (CM) utilization in pelleted calf starter mixtures, and to compare the effects of CM use in calf starter mixtures with other high-protein by-products. In Study 1, 45 female Holstein calves (44.7 ± 4.2 kg, 24.2 ± 2.8 days of age) were allocated to one of three treatments and fed a pelleted starter mixture containing: 1) soybean meal (SBM) as the main source of protein (TSBM); 2) CM as the main source of protein (TCM); or 3) CM as the main source of protein with supplemental rumen-unprotected lysine (TCML). In Study 2, 100 female Holstein calves (44.3 ± 4.8 kg, 17.7 ± 2.1 days of age) were assigned to one of four treatments and fed pelleted starter mixtures with: 1) low CM inclusion (10%; LOW); 2) low CM inclusion supplemented with feed enzymes (xylanase, glucanase, invertase, protease, cellulase, amylase, and mannanase; LOW+); 3) high CM inclusion (32%; HIGH); and 4) high CM inclusion supplemented with feed enzymes (HIGH+). In Study 3, 120 female Holstein calves (44.1 ± 4.9 kg, 18.3 ± 1.9 days of age) were allocated to one of four treatments and fed a pelleted starter mixture with: 1) moderate CM inclusion (24%; MC); 2) high CM inclusion (34%; HC); 3) moderate inclusion of extruded CM (MEC); and 4) high inclusion of extruded CM (HEC). Study 1 lasted 70 days and included 63 days of milk replacer (MR) feeding (6 L/day; 150 g of MR in 1 L) and 7 days after weaning. Studies 2 and 3 lasted 63 days and encompassed a period of MR feeding (6 L/day; 150 g MR in 1 L). In Study 1, final body weight (BW), average daily gain (ADG), starter intake, and fecal score did not differ among treatments but feed efficiency (gain to feed ratio) was greater for TSBM than for TCM and TCML. No differences between TCM and TCML were found. In Study 2, LOW+ and HIGH+ treatments had higher final BW, ADG, and starter intake while lower number of days with diarrhea. The number of days with diarrhea was also lower for high CM treatments. In Study 3, fecal score and the number of days with diarrhea was reduced when extruded CM was used in the starter mixture but BW, ADG, and feed efficiency were not different between treatments. In conclusion, rumen-unprotected lysine supplementation in a pelleted starter mixture containing CM as the main source of protein does not improve the performance of calves. Feed enzymes inclusion in calf starter can be used to increase feed intake and ADG of calves and extrusion of CM to improve fecal score of calves.

Funding

Studies were funded from internal funds of the University of Agriculture in Krakow (Kraków, Poland), from “Fundusz Promocji Roślin Oleistych” (Krajowy Ośrodek Wsparcia Rolnictwa, Warszawa, Poland), and partially supported by CBS Bio Platforms (Calgary, Canada).

Assessing the relationship between ruminal VFA synthesis and concentrations in dairy cows fed sucrose or ethanol rich diets

E.M.V. Hvas[1], M. Hanigan[2], M.R. Weisbjerg[1], M. Larsen[1]
[1]Department of Animal and Veterinary Sciences, AU Viborg, Aarhus University, Tjele, Denmark. [2]Department of Dairy Science, Virginia Tech, Blacksburg, USA (emma.hvas@anivet.au.dk)

In ruminant nutrition research, ruminal VFA concentrations serve as a proxy for ruminal VFA synthesis. This study examined rumen VFA metabolism in cows fed sugar- or ethanol-rich beet diets and the relationship between measured ruminal VFA concentrations and estimated synthesis. Sugar beets were washed, crushed, and stored either fresh (frozen at -20°C) or ensiled (barrels) in daily portions. Four rumen cannulated Danish Holstein cows were used in a replicated (one complete and one incomplete) 3×3 Latin square design with three treatments and three 21-d periods. The control diet (CON) was based on grass/clover silage, maize silage, barley, soybean meal, dried beet pulp, urea, and minerals. In the sucrose (SUC) and ethanol (ETH) rich diets, 20% of DM from CON was replaced with either fresh or ensiled beets. The diets were fed ad libitum as total mixed rations. Each period comprised 10-d of adaptation, and 11-d of collection. To estimate ruminal VFA synthesis by isotope enrichment, infusions were performed over 3 days with [^{13}C]-labelled acetate, propionate, butyrate, isobutyrate, isovalerate, or ethanol. Each tracer was infused individually at 102 mL/h for 5 h, with two sessions performed per day, starting at 0700 h and 1300 h. Ruminal fluid samples were collected hourly during infusions and 15 min before the first infusion each day and analysed for [^{13}C] enrichment using GC-C-IRMS. Ruminal VFA synthesis was computed using the FME package in R, expanding the approach described by Sutton et al. (2003) and Nolan et al. (2014) from a 3-pool to a 6-pool rumen model to include isobutyrate, isovalerate, and ethanol, along with dietary input of all VFA and ethanol. Linear regression models were used to analyse the relationship between measured ruminal concentrations and estimated VFA synthesis, with the estimated synthesis as the dependent variable and measured average concentration per treatment for cow within period as the independent variable. The r values were generally low, with the highest observed for isobutyrate and isovalerate for ETH (r=-0.900, and r=-0.841, respectively), and butyrate for CON (r=0.727). The remaining r values were ≤±0.616, with the lowest observed for acetate for ETH (r=-0.147). Additionally, no interactions were found between measured concentrations and treatment ($P \geq 0.28$, for all). There was an effect of treatment and measured concentrations for acetate and butyrate ($P \leq 0.05$, for all). Sutton et al. (2003) also observed poor relation between total production (corresponding to VFA available for absorption, excluding dietary influx) and measured ruminal concentrations of acetate and butyrate, and a stronger relationship for propionate using a 3-pool model. In conclusion, measured ruminal concentrations of VFA are generally a poor estimate of synthesis, thus caution should be taken when interpreting measured ruminal concentrations in relation to rumen metabolism.

References

Nolan, J.V., Hegarty, R.S., Longland, A.C., Pirie, F.J., Trevaskis, N.L., van der Werf, J.H.J., 2014. Animal Production Science 54, 2082-2088. https://doi.org/10.1071/AN14539

Sutton, J.D., Dhanoa, M.S., France, J., 2003. Journal of Dairy Science 86, 3620-3633. https://doi.org/10.3168/jds.S0022-0302(03)73968-X

Funding

The project was funded by The Danish Agricultural Agency (Copenhagen, Denmark; GUDP grant no. 34009-21-1845) and the Ph.D program for E. M. V. Hvas was supported by the Graduate School of Technical Sciences (GSTS, Aarhus University, Aarhus, Denmark).

Effect of feeding dried industrial hemp leaves on rumen fermentation and microbial protein production

J. Schwerdtfeger, S. Görs, B. Kuhla
Research Institute for Farm Animal Biology (FBN), Dummerstorf, Germany (b.kuhla@fbn-dummerstorf.de)

Imports of protein-rich feeds such as soya have a high carbon footprint and the use of soya as animal feed intensifies feed-food competition. The use of domestically grown protein crops, such as hemp leaves, could reduce the carbon footprint from dairy cow nutrition and does not compete with human nutrition. The objective of the study was to investigate the effects of substituting soya extraction meal with hemp leaves in dairy cow nutrition on rumen fermentation and microbial protein production. For this purpose, 12 first-lactating dairy cows received either a total mixed ration (TMR) containing 7.4% dried hemp leaves of the variety "Santhica 27" (HEMP) or a TMR with 3.5% soya extraction meal (CON) in a cross-over design. Diets were formulated isoenergetic and isonitrogenous and fed for 3 weeks separated by a 2-week washout period. Feed intake and milk yield were measured during each experimental period. On day 14 of each experimental period, a spot urine sample was taken and rumen fluid was collected using an oral stomach tubing. The ruminal pH was measured, the ruminal ammonia concentration was determined using the Conway microdiffusion method. The concentration of short-chain fatty acid (SCFA) in rumen fluid was determined via gas chromatography. High-performance liquid chromatography was used to measure urinary N metabolites. Ruminal microbial protein production (MPP, g/d) was estimated using the equation by Janicek et al. (2008), with body weight, purine derivative and creatinine concentrations as independent variables. Data were analysed using a linear mixed model in R (4.3.1, function lmer, package lmerTest), except for urinary N-metabolites where a linear model was applied. The models included group (HEMP, CON), sequence, experimental period and block as fixed effects and animal ID as random. Feeding a diet containing hemp reduced dry matter intake by 1.7 kg/d and milk yield by 1.1 kg/d ($P < 0.05$). Supplementation of hemp leaves tended to lower the ruminal pH ($P = 0.056$) but did not affect ammonia and absolute SCFA concentrations, except iso-valeric acid, which was lower in the HEMP group ($P = 0.047$). Also, the molar percentages of individual SCFA were not different between groups. The inclusion of hemp leaves reduced urinary creatine, creatinine, and urea concentrations ($P < 0.05$) and tended to reduce urinary hippuric acid and total purine concentrations ($P < 0.1$). However, urinary allantoin and uric acid concentrations as well as MPP were unaffected by the diet. In conclusion, substitution of soya extraction meal with hemp leaves has only minor effects on rumen fermentation and did not affect microbial protein production. The reduction in feed intake and milk yield argues against the use of hemp leaves under practical feeding conditions.

Reference

Janicek, B.N., Kononoff, P.J., Gehman, A.M., Doane, P.H., 2008. Journal of Dairy Science 91, 3544-3553. https://doi.org/10.3168/jds.2007-0777

Funding

This research was funded by ELER European Innovation Partnership for agricultural productivity and sustainability - project funding Mecklenburg-Western Pomerania, project No. EIP-Agri-MP-2022-BBTDÜBKW.

Effects of supplementing tree leaves from poplar on crude protein digestibility, microbial protein production and nitrogen balance of hay-fed sheep and goats

K. Helffenstein[1], M. Wilkens[2], B. Kuhla[1]
[1]Research Institute for Farm Animal Biology (FBN), Dummerstorf, Germany. [2]Leipzig University, Institute of Animal Nutrition, Nutrition Diseases and Dietetics, Leipzig, Germany (b.kuhla@fbn-dummerstorf.de)

The combination of woody plants, pasture and livestock farming, so-called silvopastoral systems, have exert beneficial effects for both the environment and the animals living in it. The leaves of trees and hedges can provide an additional source of fodder for livestock. Poplars, a tree species used for alley cropping, contain significant amounts of crude protein (CP) (1). The objective of this study was to investigate the nutritional value of poplar leaves for sheep and goats in terms of CP digestibility and microbial protein production (MPP), the latter assessed by urinary purine excretions. Four Coburger Fuchs wethers (1 year, 50 kg body weight (BW)) and four Saanen goat wethers (1.5 year, 55 kg BW) were fed either hay (HAY) or a combination of hay and 36% (sheep) or 38% (goats) dried black poplar hybrid (*P. nirga x P. deltoides* (PNIG)) leaves for 3 weeks; each animal species in a 4x4 crossover design. Every feeding period was separated by a one-week wash-out period. During the last 4 days of each feeding period, animals were individually kept in a metabolic cage to measure feed and water intake, fecal and urinary excretions. Feed, fecal, and urine samples were analyzed for nitrogen (N) using elemental analysis. Urinary N-metabolites were analyzed by high-performance liquid chromatography. MPP was calculated as described by Janicek et al. (2008): MMP (g/d) = $\{[(28 \times BW / 113.1) \times (\text{purine derivates} / \text{creatinine}) - (0.385 \times BW^{0.75})] / 0.85\} \times (70 \times 6.25) / (0.13 \times 0.83 \times 1{,}000)$ (2). The statistical analysis was performed by R Statistical Software using a linear mixed model. For each species, differences between fixed effects (feeding group and feeding period) were tested using the Tukey Kramer test. Differences were considered significant at $P < 0.05$. The daily CP intake of sheep wethers amounted to 63.2 ± 1.9 g and 87.5 ± 10.5 g and of goat wethers to 212.6 ± 18.2 g and 251.4 ± 21.6 g in the HAY and PNIG groups, respectively ($P < 0.001$). In both sheep and goat wethers, dry matter intake, water intake, CP digestibility, MMP, and N balance were comparable between PNIG and HAY feedings ($P > 0.1$). The daily urinary urea excretions were found to be significantly higher in PNIG than in HAY goats ($P < 0.05$), but not between PNIG and HAY in sheep wethers. However, the urinary excretions of allantoin and uric acid were comparable between PNIG and HAY animals. In conclusion, poplar leaves are a suitable dietary CP source for sheep and goats. The higher urea excretion in goat wethers fed PNIG can be explained by the higher dietary CP intake.

References

Rahmann, G. Ökologische Schaf- und Ziegenhaltung – 100 Fragen und Antworten für die Praxis. Institut für Ökologischen Landbau Trendhorst (OEL), Bundesforschungsanstalt für Landwirtschaft (FAL). 2007. https://literatur.thuenen.de/digbib_extern/zi043732.pdf
Janicek, B.N., Kononoff, P.J., Gehman, A.M., Doane, P.H., 2008. Journal of Dairy Science 91, 3544-3553. https://doi.org/10.3168/jds.2007-0777

Funding

We thank the Federal Ministry of Food and Agriculture (BMEL) for funding based on a resolution of the German Bundestag (281C705A21).

Improving protein quality in grass silage with biological silage additives

J. Witt[1], E. Kramer[1], J. Sprafke[2], M. Nelles[2,3]
[1]ISF GmbH, Pinneberg, Germany. [2]Department of Waste and Resource Management, University of Rostock, Rostock, Germany. [3]The German Centre for Biomass Research, Leipzig, Germany (johanna.witt@is-forschung.de)

Achieving sustainable dairy farming requires improving nitrogen (N) efficiency. A significant factor affecting N efficiency in dairy farming is the protein degradation during ensiling of forage for feed production. The use of bacterial additives in silage has been shown to reduce protein degradation during ensiling (Winters et al., 2001). Therefore, the effect of two biological silage additives on fermentation and protein quality in grass silage was tested. The forage (3rd cut, Tall fescue), with a dry matter (DM) of 30.8%, water-soluble carbohydrates of 13.4% DM, and crude protein (CP) of 13.7% DM, was ensiled in mini silos (1.5 l). Three treatments in three replications were performed: 1) CON – untreated control; 2) INO1 – inoculant with homo- and heterofermentive lactic acid bacteria (Bonsilage Speed G*, L. buchneri, L. diolivorans, L. plantarum*), application rate 250.000 CFU/g FM; 3) INO2 – inoculant with homo- and heterofermentive lactic acid bacteria (Bonsilage Fit G, *L. buchneri, L. plantarum, L. rhamnosus*), application rate 300.000 CFU/g FM. The glasses were opened after 1, 3, 5, 7, 14 and 90 days of storage at 20°C. Parameters measured included pH, NH_3-N, DM, CP, and fermentation patterns. For statistical analysis, the Mann-Whitney U-Test was used to assess significant differences ($p<0.05$) between treatments. Treated silages (INO1, INO2) showed significantly lower pH values than CON at all opening days. Likewise, NH_3-N in % fresh matter were significantly lower in the treated silages at all opening days. The significant difference between the treated silages and CON was also confirmed for NH_3-N of total N at day 14 and day 90 (INO1: 6.67% and 9.47%; INO2: 6.43% and 9.54%; CON: 10.9% and 14.1%). Lactic acid, acetic acid, and 1,2-propanediol were higher in the treated silages (INO1, INO2), while butyric acid levels were significantly lower in INO1 (0.03% DM) and INO2 (0.00% DM) compared to CON (0.74% DM) at day 90. These results suggest that the treatment of the biological additive (INO1, INO2) reduced protein degradation during the silage fermentation process by accelerating pH reduction, which inhibited protease activity and suppressed the growth of undesirable protein-degrading bacteria (Slottner and Bertilsson, 2006). The decrease in protein degradation is confirmed by the significantly lower NH_3-N in the treated silages, as NH_3-N is used as an indicator of protein degradation evaluation. In contrast, the lower pH reduction and the presence of butyric acid in the CON suggest protein degradation by Clostridia. In conclusion, this study presents the positive effects of the biological additives (INO1, INO2) on the fermentation and protein quality of grass silage. These treatments improve nitrogen efficiency of forage production and are therefore a potential strategy to save resources and could play an important role in strategies for sustainable milk production.

References

Slottner, D., Bertilsson, J., 2006. Animal Feed Science and Technology 127, 101-111. https://doi.org/10.1016/j.anifeedsci.2004.10.007

Winters, A.L., Fychan, R., Jones, R., 2001. Grass and Forage Science 56, 181-192. https://doi.org/10.1046/j.1365-2494.2001.00265.x

Effect of feeding wet toasted fava beans in two forage situations on milk production in dairy cows

W. Wang, M. Tax, M. Johansen, M.R. Weisbjerg, M. Larsen
Aarhus University - Research Center Foulum, Tjele, Denmark (Mogens.Larsen@anivet.au.dk)

On-farm, wet toasting could be an alternative to regular drying legume grains, giving a dual-purpose of toasting. Wang et al. (2025) concluded a greater reduction potential of effective rumen protein degradation by toasting fava beans in moist condition compared with toasting in dry condition. The objective in the current study was to investigate the effect of feeding wet toasted fava beans (Vicia faba) in two forage situations on lactational performance in dairy cows. Forty-eight Danish Holstein cows (24 primi- and 24 multiparous) were used in twelve 4×4 Latin square designs (blocks) with 4 periods of 21 days. The 4 dietary treatments were organized in a 2×2 factorial arrangement with 2 fava bean processing (untoasted vs. wet toasted; 23.6% of diet DM) and 2 forage situations (0.75:0.25 vs. 0.25:0.75 grass-clover:maize silage ratios in 60% diet forage DM). One batch of fava beans was subdivided into two of which one was wet toasted after hydrating to moisture content of 207 g/kg (soaking water in vertical mixer for 30 min followed by 16 h on floor). After hydration, fava beans were toasted (flame tumble toaster; 3 min retention time and 125℃ exit temperature). Both types of fava beans were ground (3-mm screen). Cows were fed ad libitum in automated feed bins, milked twice daily, and milk was sampled weekly. Data obtained in the last week of each period was averaged and used in data analysis. Data was analysed using R with a model including fixed effects of block, period, wet toasting, forage situation, interaction between toasting and forage, and cow as a random effect. Dry matter intake was lower with wet toasted compared with untoasted fava beans (21.8 vs. 22.2 ± 0.34 kg/d; $P = 0.01$). The ECM yield was greater with wet toasted compared with untoasted fava beans (31.0 vs. 30.5 ± 0.36 kg/d; $P = 0.01$). Milk protein yield did not differ between wet toasted or untoasted fava beans ($P = 0.43$), due to lower protein content with wet toasted fava beans (37.3 vs. 37.8 ± 0.03 g/kg; $P < 0.01$). Milk urea content did not differ between wet toasted or untoasted fava beans ($P = 0.26$). Gross feed efficiency (ECM/DMI) was greater with wet toasted compared with untoasted fava beans (1.51 vs. 1.46 ± 0.02 kg/kg; $P < 0.01$). Body weight and condition score did not differ between wet toasted or untoasted fava beans ($P \geq 0.30$). No interactions to forage situation were observed for these variables ($P \geq 0.15$) indicating that the obtained effect of wet toasting fava beans was independent of high or low grass silage inclusion. In conclusion, feeding wet toasted compared to untoasted fava beans gave a greater feed efficiency independent of forage situation.

Reference

Wang, W., Aljundi, A., Hansen, N.P., Weisbjerg, M.R., Lund, P., Larsen, M., 2025. Animal Feed Science and Technology 323, 116281. https://doi.org/10.1016/j.anifeedsci.2025.116281

Funding

The project was funded by The Danish Milk Levy Fund (Aarhus, Denmark).

Effects of flint corn processing method and urea source on blood parameters of Nellore heifers fed high-concentrate diets

R.A. Lino[1], G.P. Silva[1], I.G. Fávero[1], S.P. Souza[1], A.C.S. Santos[1], I.P.C. de Carvalho[2], J. Martín-Tereso[2], E.D. Batista[1]

[1]Universidade Federal de Lavras, Lavras, Brazil. [2]Trouw Nutrition R&D, Boxmeer, Netherlands (erick.batista@ufla.br)

In ruminants, manipulating the site of urea absorption in the body can be an effective strategy to reduce ammonia peaks in the rumen and bloodstream due to the high hydrolysis rate of urea used in feeding. In this sense, evaluation of blood parameters can provide information on metabolism related aspects, such as the blood urea pool, glucose levels, and the activity of enzymes relative to liver health. The objective of this study was to evaluate the interaction between flint corn processing methods and urea source on blood characteristics of Nellore heifers fed finishing diets. Eight rumen-cannulated Nellore heifers (321 ± 15.2 kg of body weight and 12 ± 2.0 months old) were distributed in a replicated 4 × 4 Latin square design, balanced for residual effects. Treatments were arranged in a 2 × 2 factorial design; 2 corn grain processing methods [CPM; dry ground (DGC) and rehydrated and ensiled (REC)] were combined with urea (U) and coated urea (CU). The CU is a product that aimed to partially shift urea supply to the post-ruminal gastrointestinal tract (82% of urea and 18% of palm oil on DM basis; 72.8% of ruminal protection and 92.9% digestibility), according to Rauch et al. (2023). The diet consisted of 72% corn-based concentrate and 28% Shredlage®. The experiment lasted 104 days, divided into four 26-day periods. Each experimental period included 14 days of diet adaptation and 12 days of data collection. Blood samples were collected every 10 hours between days 16 and 20, totaling 12 samples collected each 2 h. There were CPM × urea sources interactions effects on plasma urea-N (PUN) and serum AST concentrations ($P < 0.05$). Heifers fed DGC+CU presented greater PUN levels than those fed DGC+U (17.0 vs 15.1 mg/dL), but there was no difference when REC-diets were offered (averaging, 14.3 mg/dL). In the opposite way, serum AST concentration was lower in heifers fed DGC+CU than DGC+U ($P < 0.05$; 51.9 vs. 58.5 mg/dL), while it was not affected when REC-diets were fed ($P > 0.05$; in average, 28.0 mg/dL). Overall, results did not indicate statistical differences for IGF-1 (223 ng/mL), creatinine (1.24 mg/dL), alkaline phosphatase (236 U/L), aspartate transaminase (18.5 U/L), γ-glutamil transferase (16.0 U/L), total protein (6.7 mg/dL), albumin (2.7 mg/dL), and globulin (3.9 mg/dL) serum concentrations. Plasma glucose levels were greater in heifers fed REC-diets ($P < 0.001$; 79.7 vs. 76.8 mg/dL). In conclusion, CPM and US promoted little effect on blood characteristics of Nellore heifers fed corn-based diets.

Reference

Rauch, R., Nichols, K., de Carvalho, I.P.C., Daniel, J.B., Martín-Tereso, J., Dijkstra, J., 2023. Journal of Animal Physiology and Animal Nutrition 109, 64-75. https://doi.org/10.1111/jpn.14034

Funding

This study was funded by Trouw Nutrition (Boxmeer, the Netherlands). The authors would like to thank FAPEMIG, CNPq, and NEPEC for their support.

Relationship between nutritional components and fermentation characteristics of common herbs

M.A. Shuva[1,2], M. Hünerberg[1,2], J. Hummel[1,2]
[1]Department of Animal Sciences, Division of Ruminant Nutrition, University of Göttingen, Göttingen, Germany. [2]Centre of Biodiversity and Sustainable Land Use (CBL), Göttingen, Germany (jhummel@gwdg.de)

Fiber and carbohydrate composition, including cellulose, hemicellulose, and lignin, influence fermentation characteristics and the metabolizable energy (ME) content of forages. Understanding these relationships is important to predict their nutritional quality. This study investigates the correlations between nutrient contents like carbohydrate/fiber fractions and fermentation parameters in herbs, as a forage group recently receiving increased attention due to their resilience to various impacts of climate change (e.g., more frequent episodes of extreme heat, increased drought risk). A total of 32 samples from seven herb species (*Lotus corniculatus* (7 accessions), *Medicago lupulina* (4), *Plantago lanceolata* (7), *Achillea millefolium* (4), *Cichorium intybus* (4), *Carum carvi* (4), and *Hedysarum coronarium* (2)) were analyzed. Samples were freeze-dried and analyzed for proximate composition, NDFom, ADFom, and ADL (Van Soest et al., 1991); hemicellulose, cellulose and non-fiber carbohydrates (NFC) were calculated. In vitro gas production (GP) was measured using the Hohenheim gas test (Menke and Steingass, 1988). The GP rate and maximal GP (GPmax) were estimated using an exponential model. ME was estimated using equation 12f of [2]. Correlation and regression analyses were conducted (R Studio, v4.3.3), to assess relationships among nutrient contents and fermentation parameters. Significance was declared at $p<0.05$. *Medicago lupulina* had the highest crude protein (CP; 162 ± 34 g/kg DM) and ME (10.7 ± 0.5 MJ/kg DM), while *Plantago lanceolata* had the lowest CP (65 ± 8 g/kg DM) and *Achillea millefolium* the lowest ME (7.4 ± 0.4 MJ/kg DM). *Lotus corniculatus* had the highest NDFom (525 ± 28 g/kg DM), and *Carum carvi* the lowest (303 ± 14 g/kg DM). GP was highest in *Cichorium intybus* (49.7 ± 3.9 mL/200 mg DM) and lowest in *Achillea millefolium* (32.3 ± 1.5 mL/200 mg DM). NFC correlated positively with gas production at 24h (GP_24h) ($r=0.71$, $p<0.01$), GP ($r=0.56$, $p<0.05$), and GPmax ($r=0.72$, $p<0.01$). In contrast, cellulose correlated negatively with GP_24h ($r=-0.81$, $p<0.001$), GP rate ($r=-0.79$, $p<0.001$), and ME ($r=-0.76$, $p<0.001$). Lignin also reduced fermentation, while hemicellulose and CP had weak, non-significant impacts. Regression analysis confirmed cellulose as main component reducing GPmax ($\beta=-0.0718$, $p<0.001$, $R^2=0.616$) and GP rate ($\beta=-0.0003$ $p<0.001$, $R^2=0.732$). Hemicellulose had a slightly negative effect on GP rate ($\beta=-0.0003$, $p=0.037$), while CP showed a weak positive trend ($p=0.070$). This study demonstrates the significant impact of carbohydrate composition on the fermentation characteristics of herbs. Cellulose and lignin reduced GP, while NFC enhanced it, improving digestibility. Regression analysis confirmed cellulose as strongest negative predictor, while the hemicellulose content only had minor impact. Optimizing fiber composition, e.g. by choosing adapted varieties and an optimal harvest date, can greatly enhance the feeding value of herbs.

References

Van Soest, P.J., Robertson, J.B., Lewis, B.A., 1991. Journal of Dairy Science 74, 3583-3597. https://doi.org/10.3168/jds.S0022-0302(91)78551-2
Menke, K.H., Steingass, H., 1988. Animal Research and Development 28, 7-55.

Funding

We would like to thank the German Federal Ministry of Food and Agriculture (BMEL) for funding this project.

Nanoemulsified corn oil in lactating Barki nutrition: Effect on intake, nutrient digestibility, rumen fermentation characteristics, and microbial population

M. Gao[1,2], R. Li[1], M.S.A. Khattab[3], A.M. Abd El Tawab[3], Y. Liu[1,2,4], M. El-Sherbiny[3,5]
[1]State Key Laboratory of Reproductive Regulation and Breeding of Grassland Livestock, Inner Mongolia University, Hohhot, China. [2]National Sheep Genetic Evaluation Center, Inner Mongolia University, Hohhot, China. [3]Department of Dairy Science, National Research Centre, Giza, Egypt. [4]Department of Animal Genetics, Breeding, and Reproduction, College of Animal Science, Inner Mongolia Agricultural University, Hohhot, China. [5]Institute of Animal Nutrition, Nutrition Diseases and Dietetics, Faculty of Veterinary Medicine, Leipzig, Germany (elsherbiny.nrc.eg@gmail.com)

Feeding oils to ruminants enhances energy intake, milk fat composition, and reproduction. Vegetable fats provide essential fatty acids that improve animal fertility and boost productivity. However, diets rich in polyunsaturated fatty acids can affect nutrient digestibility and reduce milk fat in ruminants. Notably, fat in whole oilseeds is more ruminally inert than free oil due to slower fat release (Ibrahim et al., 2021). That is why it is crucial to develop alternative forms of oil supplementation, primarily to preserve the benefits of vegetable oils while mitigating the adverse effects of incorporating them as an ingredient in animal feed. Our team has been studying the potential of oil nanoemulsions on rumen fermentation, milk productivity, and composition (El-Sherbiny et al., 2023). However, the effect of nanoemulsified oil supplementation on in vivo rumen fermentation patterns, nutrient intake, and microbial population is not well-documented. In this study, the nanoemulsified oil form was tested following an in vivo trial on 21 lactating dairy ewes in early lactation randomly allocated to the following treatments: Control – a basal diet consisting of 50% concentrate mixtures and 50% berseem clover without any supplementation, CO – the control diet + corn oil supplied at 3% on a dry matter basis, NCO – the control diet + nanoemulsified corn oil provided at 3% on dry matter basis. A completely randomized design, lasting 30 days (25 days of adaptation and 5 days of sampling), was employed with seven ewes in each treatment. The PROC MIXED procedure of SAS® was used to analyze all data using a model that included a fixed effect of treatment and a random effect of the ewe. The multiple comparison Tukey tests were used to evaluate differences among the treatments. Based on the results, despite feeding oil according to the recommended values, CO decreased the dry matter intake by 8.3% and 6.7% compared to the Control and NCO, respectively. The negative impact of CO extended to reduce the concentrations of ammonia and total volatile fatty acids in the rumen. On the other hand, NCO had less effect on the biohydrogenation of fatty acids compared to CO; noticeably, higher proportions of unsaturated fatty acid (UFA) were associated with NCO; these results were supported by an increase in the rumen microbial population with NCO compared to CO, especially of the biohydrogenation bacteria, *Butyrivibrio fibrisolvens* and *Butyrivibrio proteoclasticus*, which showed higher abundance with NCO despite the low presence of biohydrogenation intermediates. In conclusion, NCO demonstrated the ability to decrease the transformation of unsaturated fatty acids into saturated fatty acids in the biohydrogenation environment, while mitigating the toxic effects of the accumulated unsaturated fatty acids on rumen bacteria. This effect was not associated with decreased dry matter intake, changes in nutrient digestibility, or the fermentation patterns.

References

Ibrahim, M.I., Galal, A.G., Elkomy, A.E., Zeitoun, M.M., 2021. Tropical Animal Health and Production 53, 422. https://doi.org/10.1007/s11250-021-02863-4

El-Sherbiny, M., El-Said, K.S., El-Wahed, R.A., Ebrahim, H.Y., 2023. Molecules 28, 358. https://doi.org/10.3390/molecules28010358

Funding

We greatly appreciate the Alexander von Humboldt Foundation's support of Dr. Mohamed El-Sherbiny through the Georg Forster Research Fellowship Programme for Experienced Researchers.

Effect of a milk replacer supplementation with monoglycerides on performance and gut development in calves

G. Dusel[1], S. Ped[1], E. von Heimendahl[2], A. Barri[2], T. Scheu[3], J. Hayer[3], C. Koch[3]
[1]University of Applied Sciences, Bingen, Germany. [2]BASF SE, Ludwigshafen, Germany. [3]LVAV Hofgut, Neumühle, Germany (g.dusel@th-bingen.de)

Calves are exposed to various stressors related to nutrition, transportation, and changes in their housing environment. These stressors can lead to health issues, impaired gut development, and reduced performance. This study investigated the effects of a monoglyceride (MG) blend of short- and medium-chain fatty acids (MCFA) on the health status, performance, and intestinal morphology of calves. A total of 24 male Simmental calves (starting at 28 days of age) were divided into two groups and housed in a fresh-air stable with two identical compartments on straw bedding. They received a commercial high-quality milk replacer either without (C, control) or with an additional MG blend of short- and medium-chain fatty acids (5 g/animal/day) administered via a medicine dispenser. Milk replacer intake was recorded daily per animal using a transponder system. A dry total mixed ration (TMR) was offered daily to all calves, and consumption per group was measured by weighing feed residues. Fresh water and hay were available ad libitum via an automatic water dispenser. On day 35, four animals per treatment were euthanized for morphological analysis of ileal and jejunal tissue samples. Supplementation did not affect calf health but resulted in a significantly higher body weight in the MG group compared to the control at week 3 (C: 88.5 kg vs. MG: 92.8 kg; $P = 0.040$) and a numerically higher weight at the end of the trial (C: 127.4 kg vs. MG: 133.6 kg). No significant differences were found in total energy intake between the groups ($P = 0.976$). MG supplementation showed a trend toward increased villus width (C: 0.12 µm vs. MG: 0.19 µm, $P = 0.072$) and villus surface area (C: 0.19 µm vs. MG: 0.35 µm, $P = 0.055$) in the jejunum, indicating a greater absorptive area, which could explain the improved performance observed in this study. Butyrate, supplied as Monobutyrin via the MG blend, is known to influence immune function and inflammatory response (Zhong et al., 2023), potentially leading to energy and protein savings in the MG group. Additionally, along with Monobutyrin, MCFA monoglycerides may have contributed to a beneficial gut microbiota, further enhancing performance (Batovska et al., 2009). In summary, supplementing a specific MG blend may support intestinal development and growth performance in calves, even when they are already fed a high-quality milk replacer.

References

Batovska, D.I., Todorova, I.T., Tsvetkova, I.V., Najdenski, H.M., 2009. Polish Journal of Microbiology 58, 43-47.
Zhong, H., Yu, W., Wang, M., Lin, B., Sun, X., Zheng, N., Wang, J., Zhao, S., 2023. Animal Nutrition 14, 88-100. https://doi.org/10.1016/j.aninu.2023.04.004

Impact of vitamin A source and supplementation levels on receptor activator of nuclear factor kappa-B ligand and osteoprotegerin expression in broiler tibia

P. Sacakli[1], A. Calik[1], A. Ceylan[1], M. Shazaib Ramay[1], Ö. Özgenç Çınar[1], D. Özen[1], W. Pelletier[2], A. Barri[2], Y. Shastak[2]
[1]Ankara University, Ankara, Turkey. [2]BASF SE, Ludwigshafen am Rhein, Germany (yauheni.shastak@basf.com)

This study evaluated the impact of various sources and levels of vitamin A supplementation on the expression of RANKL (Receptor Activator of Nuclear Factor Kappa-B Ligand) and OPG (Osteoprotegerin) in broiler tibia. Vitamin A is essential for bone metabolism, regulating these proteins, which influence bone resorption and formation (Jacobson et al., 2004). RANKL promotes osteoclast activity, increasing bone resorption, while OPG inhibits RANKL, reducing bone loss. The RANKL/OPG ratio reflects bone turnover, with higher ratios indicating greater resorption and lower ratios favoring bone formation. A control diet, based on wheat-soybean meal and formulated per Aviagen (2022) guidelines but without added vitamin A, was compared to diets supplemented with 2000 or 4000 IU/kg of five vitamin A sources (products A-E), all supplied as beadlet powders containing a minimum of 1,000,000 IU/g of vitamin A). A total of 1,287 Ross 308 male broiler chicks were assigned to these experimental diets for 23 days. Chicks were housed in floor pens (110 × 80 cm) with wood shavings, with nine pens per treatment and 13 birds per pen. Body weight gain (BWG) and feed intake (FI) were recorded on day 23. On day 23, right tibia samples ($n = 9$ per treatment group) were fixed in 10% neutral buffered formalin and decalcified in 5% formic acid for five weeks. After paraffin embedding, 7 µm sections were prepared, deparaffinized, rehydrated, and subjected to antigen retrieval. Sections were incubated overnight at 4°C with primary antibodies against RANKL and OPG, followed by biotinylated secondary antibody and streptavidin-HRP. Immunolabeling was visualized using DAB chromogen, with Gill's hematoxylin counterstaining. Data were analyzed using the ANOVA procedure in SPSS (version 14.01), and a general linear model in SAS was applied for simultaneous linear regression. The results indicated that supplementation with products A, B, C, and D at both 2000 and 4000 IU/kg significantly improved ($P < 0.001$) BWG and feed conversion ratio (FCR) compared to the control diet and product E. The effect of vitamin A supplementation on RANKL and OPG levels was nonlinear, preventing direct comparison of sources via linear regression. Only products A, D, and E at 4000 IU/kg significantly reduced the RANKL/OPG ratio (1.34, 1.33, and 1.60, respectively) compared to the control group (2.44, $P < 0.001$), suggesting a shift towards promoting bone formation. Compared to liver retinol levels, which were also assessed in this trial and showed a clear linear response to vitamin A supplementation (as reported by Sacakli et al., 2025), the RANKL/OPG ratio appeared to be less sensitive in evaluating the impact of vitamin A supplementation. Additional research is needed to further clarify its role in bone metabolism in poultry.

References

Jacobson, A., Johansson, S., Branting, M., Melhus, H., 2004. Biochemical and Biophysical Research Communications 322, 162-167. https://doi.org/10.1016/j.bbrc.2004.07.092

Sacakli, P., Calik, A., Ramay, M.S., Özen, D., Pelletier, W., Gordillo, W., Shastak, Y., 2025. Comparative evaluation of relative bioavailability among various sources of vitamin A in broilers. The 24th European Symposium on Poultry Nutrition, Maastricht, the Netherlands (accepted).

Effects of nitrogen and phosphorus reduction on the amino acid profile in the rumen of young goats

K.W. Wittenberg[1], M.B. Burmester[1], K.H. Hustedt[1], N.S. Schnepel[1], C.V. Visscher[2], A.S.M.-B. Muscher-Banse[1]

[1]Institute for Physiology and Cell Biology, University of Veterinary Medicine Hannover, Hannover, Germany. [2]Institute for Animal Nutrition, University of Veterinary Medicine Hannover, Hannover, Germany (alexandra.muscher@tiho-hannover.de)

Reducing nitrogen (N) and phosphorus (P) in ruminant diets lowers feed costs, conserves resources, and reduces environmental impact by minimizing N and P emissions. Dietary composition significantly influences microbial activity in the rumen, particularly amino acid (AA) homeostasis. While previous studies have examined microbial protein synthesis and N efficiency in response to dietary N or P reduction, data on alterations in the ruminal AA profile under combined N and P restriction are scarce, particularly in young goats. Ruminal microbes degrade and synthesize both essential (EAA) and non-essential amino acids (NEAA), making their balance critical for protein efficiency in low-N and low-P diets. Recent studies suggest that dietary N and P restrictions affect not only microbial activity but also systemic metabolic pathways in the liver, influencing AA sensing (Weber et al. 2023). However, the impact on ruminal AA composition remains unclear. This study aimed to determine the effects of reduced dietary N and/or P on the ruminal AA profile of young goats, providing insights into microbial metabolic adaptations. Twenty-eight male Colored German goats (10 weeks old) were assigned to one of four feeding groups (n = 7 per group), receiving diets with following crude protein (CP) and P contents (percentage of dry matter): control (16.5% CP; 0.5% P), N-reduced (8.4% CP; 0.5% P), P-reduced (16.9% CP; 0.1% P), and N- and P-reduced (8.1% CP; 0.1% P). All diets were isoenergetic and fed for six weeks. Ruminal fluid samples were collected post-mortem. Concentrations of urea and phosphate (Pi) were determined using standard photometric techniques whereas short-chain fatty acids (SCFA) were quantified by gas chromatography. The AA profile was analysed via ion-exchange chromatography. Data were analysed using two-way ANOVA. N reduction significantly decreased urea concentration in ruminal fluid, while Pi concentrations were lower in the P-reduced feeding groups as expected. SCFA concentrations were also influenced by the dietary treatments. N reduction resulted in decreased concentrations of both NEAA (e.g., alanine and glutamic acid) and EAA (e.g., threonine or lysine) in ruminal fluid. In contrast, P reduction had no significant effect on either NEAA or EAA concentrations. These findings indicate that N reduction alters the ruminal amino acid profile by decreasing both NEAA and EAA concentrations, whereas P reduction does not appear to impact AA composition. The study provides valuable insights into microbial metabolic responses to nutrient restrictions in young goats, contributing to a better understanding of ruminal adaptation mechanisms under limited dietary N and P supply. While this study focused on the ruminal AA profile, future work should include direct quantification of microbial protein synthesis to complement and further validate the observed changes in AA concentrations under dietary N and P restriction.

Reference

Weber, S.L., Hustedt, K., Schnepel, N., Visscher, C., Muscher-Banse, A.S., 2023. International Journal of Molecular Sciences 24, 7153. https://doi.org/10.3390/ijms24087153

Degradation of vitamin B_{12} in in vitro rumen fermentation

B. Hou, S. Zhang, M. Fan, M. Li, G. Zhao
China Agricultural University, Beijing, China (zhaogy@cau.edu.cn)

Vitamin B_{12} (VB_{12}) is an essential vitamin for the metabolism of animals. In adult ruminants, the ruminal microorganisms are able to synthesize VB_{12}. The VB_{12} supply from rumen microorganisms may not be enough for the requirement of ruminants. Supplementation with rumen-protected VB_{12} improved the lactation performance in dairy cows (Wang et al., 2024). However, rumen-protected VB_{12} is more costly than unprotected VB_{12}. The objective of the experiment was to study the ruminal degradability of VB_{12} using in vitro rumen fermentation technique. The first step of the two-step digestion technique of Tilley and Terry (1963) was used for in vitro rumen fermentation. Three adult beef cattle fed with a total mixed ration mainly composed of whole-corn silage, corn grain and soybean meal were used as donors of rumen fluid. An aliquot of 500 mL rumen fluid was taken from each steer two hours after morning feeding. The rumen fluid samples from the steers were well mixed and filtered through four layers of surgical gauze. Sixty centrifugation tubes, each with 50 mL in volume, were used as incubation vessels. Each tube was added with 0.4000 g air-dried feed mixture mainly composed of whole-corn silage, oat hay, corn grain and soybean meal as the main substrate. The tubes were divided into control group and treatment group. Each tube of the treatment group was added with 0.0020 g VB_{12} (purity 99%) while the control group was not. The tubes were incubated in an incubator at 39℃. Five tubes were taken out from each group to terminate the incubation in ice packs at 2, 4, 8, 12, 24, 48 h of incubation, respectively. The incubation contents in the tube were freeze-dried and used for VB_{12} analysis on HPLC. The results showed that the degradation rates (%) of VB_{12} were 28.00 ± 1.74, 31.84 ± 2.66, 37.59 ± 4.42， 48.69 ± 1.93, 55.94 ± 3.71 and 69.24 ± 2.38 at 2、4、8、12、24、48 h of incubation, respectively, linearly increased with incubation time ($P < 0.001$). Although VB12 was hydrolysable in rumen fermentation, a considerable amount of VB_{12} was not hydrolyzed. Supplementation with unprotected VB_{12} to adult ruminants could be considered based on the rumen-by pass rate and the cost of rumen-protected VB_{12}.

References

Tilley, J.M.A., Terry, R.A., 1963. Journal of the British Grassland Society 18, 104-111. http://dx.doi.org/10.1111/j.1365-2494.1963.tb00335.x

Wang, C., An, J., Bu, L., Liu, Y., Liu, Q., Guo, G., Zhang, J., Zhang, Y., 2024. Journal of Animal Physiology and Animal Nutrition 108, 635-645. https://doi.org/10.1046/j.1365-2362.1997.2000747.x

Revisiting gnotobiotic research to advance ruminant microbiome science and sustainable livestock production

F. Batistel, S.U. Khan
University of Florida, Gainesville, USA (fernandabatistel@ufl.edu)

Meeting the demand for safe and nutritious ruminant products for a global population projected to reach 9.7 billion by 2050 presents a growing challenge. Simply increasing animal numbers is not a viable solution due to limited land, resource constraints, and concerns over greenhouse gas emissions. Instead, efforts should focus on improving production efficiency and reducing the carbon footprint of livestock systems. In this context, gut microbial communities — particularly those in the rumen — offer promising solutions, given their central role in ruminant nutrition and physiology. Over the past two decades, advances in molecular techniques have significantly improved our understanding of rumen microbial composition, addressing questions like "Who is there?" and "What are they doing?". However, to fully uncover causal relationships between the host and its ruminal microbes, more controlled experimental approaches are needed. One promising avenue is the reintroduction of ruminant gnotobiotic research, which involves studying animals in fully defined microbial environments — either germ-free or colonized with specific microbes or microbial communities. Thus, this review aims to a) Identify the apparatus and techniques used to generate and maintain gnotobiotic ruminants and b) Highlight key findings and research gaps to guide future research using this approach. A comprehensive literature search was conducted using relevant keywords across bibliographic databases. After initial screening, 46 research articles and one dissertation were examined in detail. Three techniques for generating germ-free animals have been described: hysterectomy (the most widely used method), cesarean section, and natural birth followed by antibiotic treatment and isolation. Both hysterectomy and cesarean section require specialized equipment for success. The most used husbandry isolators consist of flexible film enclosures within a pen. Autoclaving and gamma irradiation have been successfully used for feed sterilization; however, vitamin supplementation is necessary to compensate for degradation caused by autoclaving. Several studies have reported successful weaning of animals through a transition from liquid to solid feed, in conjunction with inoculation of a simplified microbial community (6–8 bacteria). The bulk of research on gnotobiotic ruminants was conducted between the 1970s and 1990s, demonstrating the role of microbes in gastrointestinal tract development and key immune functions. This approach was also used to study the establishment of key ruminal bacteria and the interactions between bacteria, protozoa, and fungi. Notably, many of these past experiments used a limited number of experimental units, and the microbiological and immunological techniques available at the time were relatively basic. The reintroduction of gnotobiotic research, combined with modern molecular techniques, presents an opportunity to address fundamental questions about host-microbe interactions. Advances in this area could enhance ruminant production efficiency while also providing strategies to mitigate the environmental impact of livestock systems.

Digital twin architecture with agent-based modeling for optimal energy and protein utilization monitoring in beef systems

K. Kaniyamattam[1], K.B. Athina[2], T.T.T. La[3], V. Kulangara Veettil[1], J. Tao[2,3]
[1] Department of Animal Science, College of Agriculture & Life Sciences, Texas A&M University, College Station, Texas, USA. [2] Institute of Data Science, Texas A&M University, College Station, Texas, USA.
[3] College of Performance, Visualization & Fine Arts, Texas A&M University, College Station, Texas, USA
(karun.kaniyamattam@ag.tamu.edu)

In the United States (U.S.), beef production and consumption accounts for roughly 3.7% of the total national Green House Gas (GHG) emissions (~250 Teragram Carbon Dioxide Equivalent (Tg). Improved efficiencies over the past 50 years have reduced U.S. beef-related GHG emissions by 21% (from 324 to 255 Tg), while simultaneously producing 20% more beef with 15% fewer cattle. This study employs a digital twin of the McGregor Research Center, Texas; a real-time, high-fidelity virtual replica that integrates live sensor data with advanced computational models to monitor, predict, and ultimately reduce GHG while improving animal performance. Real-time data streams obtained through Precision Livestock Technologies (PLTs) including GreenFeed, GrowSafe, Smart Waterer, and TruTest systems capture continuous metrics such as methane emission, feed intake, water consumption and body weight dynamics, providing a framework for targeted interventions and improved nutrient utilization. Our digital twin leverages an agent-based model (ABM) built in Python using the Mesa framework to simulate a beef herd comprising hundred cattle agents. Each cow is represented with unique state variables and decision-making logic based on rule-based thresholds derived from Cattle Value Discovery System Model. Real-time sensor data streamed every minute from PLTs are integrated into the simulation, updating each agent's state and ensuring that the digital twin mirrors the real animal performance metrics. Data assimilation is achieved via a Message Queuing Telemetry Transport (MQTT) messaging protocol, which supports low-latency transmission of compact JSON payloads, enabling asynchronous processing in both Python and Unreal Engine Blueprints Visual Scripting. In Unreal Engine, a detailed farm layout modeled in Autodesk Maya is used to create a spatially accurate environment where each cow is visualized as an animated 3D avatar. Real-time updates are managed by Unreal Engine's Blueprints, which process MQTT messages to dynamically adjust the statuses of cow models, and to update a data-driven user interface that displays individual cow's metrics. The system's performance was benchmarked by measuring key metrics such as simulation step time, data transmission latency, and rendering frame rate. Verification of the ABM was conducted to ensure internal consistency, while validation involved comparing simulated performance metrics such as feed intake and weight changes to empirical data. Sensitivity analyses further confirmed that variations in parameters like feed intake yield biologically plausible individual changes in metabolism of energy, protein and methane emission. In the future we will extend this framework by integrating advanced machine learning algorithms to enhance anomaly detection and predictive capabilities within the ABM, as well as by exploring edge computing solutions to further reduce latency in data assimilation. The direction of future studies aims to establish a robust and scalable platform that bridges real-time sensor data with computational models, ultimately advancing the precision management of beef production systems.

References

Mu, M., Zhou, Y., Wu, D., 2022. Procedia Computer Science 214, 1182-1189. https://doi.org/10.1016/j.procs.2022.11.294
Zhang, Y., Zhang, Y., Gao, M., Dai, B., Kou, S., Wang, X., Fu, X., Shen, W., 2023. Computers and Electronics in Agriculture 214, 108181. https://doi.org/10.1016/j.compag.2023.108181

Funding

Texas A&M Department of Animal Science, AgriLife Research, Texas A&M Institute of Data Science

Two-dimensional transwell model for assessing nutrient uptake in intestinal organoids in pigs divergent in feed efficiency

E.G.T. van der Valk[1], L. Kruijt[1], A.A.C. de Wit[1], D.A.L. Flossdorf[1], J.H. Soellner[2], S. Verstringe[3], S.K. Kar[1], E.D. Ellen[1]
[1]WUR, Wageningen, Netherlands. [2]Topigs Norsvin, 's-Hertogenbosch, Netherlands. [3]NuScience, Drongen, Belgium (elianne.vandervalk@wur.nl)

Feed efficiency (FE) and gut health in pigs are complex traits with unclear biological mechanisms. Pig-derived intestinal organoids serve as reductionist *in vitro* models to explore these mechanisms. To study gut health, we are developing a co-culture model with immune cells, while for FE, we are establishing *in vitro* models of nutrient absorption. In this study, we developed a two-dimensional porcine intestinal organoid monolayer, using a transwell system, to assess nutrient absorption. We successfully optimized the culture conditions and established a reproducible, confluent monolayer of jejunum and ileum organoids within three days, maintaining stability for at least 24 hours. Monolayer integrity was verified through trans-epithelial electrical resistance (TEER) measurements ($\geq 400\ \Omega\cdot\text{cm}^2$) and a FITC-Dextran (4 kDa) permeability assay. qPCR analysis was performed to characterize jejunum organoids and assess their physiological relevance by comparing enterocyte marker *Villin* and dipeptide transporter *Slc15a1* expression with that of native jejunum tissue. Relative gene quantification (RQ) analysis of the enterocyte marker *Villin* revealed no significant differences ($p = 0.28$) between jejunum organoids (RQ = 1.97×10^{-3}) and jejunum tissue (RQ = 1.45×10^{-3}). Dipeptide transporter *Slc15a1* expression varied significantly across culture conditions. Organoids cultured for one day in growth medium before switching to differentiation medium had the lowest expression (Group A: RQ = 7.43×10^{-5}). Extending monolayer culture in differentiation medium for three days significantly increased expression (Group B: RQ = 2.84×10^{-4}, $p = 0.03$ vs. Group A), reaching levels comparable to jejunum tissue (Group C: RQ = 2.83×10^{-4}, $p = 0.02$ vs. Group A, $p = 0.86$ vs. Group B). These findings indicate that prolonged differentiation enhances *Slc15a1* expression in jejunum organoids to intestinal tissue levels. We aim to validate this model through follow-up experiments to evaluate nutrient transport in organoids. Altogether, our results establish a well-characterized two-dimensional intestinal organoid model with the potential for studying nutrient transport, with gene expression patterns resembling those of intestinal tissue. This two dimensional organoid model may therefore serve as a valuable system for evaluating FE in pigs. Together with the co-culture model with immune cells immune co-culture, the two-dimensional organoid model offers potential applications in livestock breeding strategies aimed at improving nutrient utilization and overall animal performance.

Ring studies for quantifying the degree of variance of the rumen simulation technique (RUSITEC)

F. Klevenhusen[1], G. Breves[2]
[1]University of Kassel, Faculty of Organic Agricultural Sciences, Section Environmentally Sustainable Animal Nutrition, Kassel, Germany. [2]University of Veterinary Medicine Hannover, Foundation, Institute for Physiology and Cell Biology, Hannover, Germany (Gerhard.Breves.iR@tiho-hannover.de)

The RUSITEC has become well established as a semi-continuous method for studying quantitative parameters of rumen microbial metabolism under in vitro conditions (Czerkawski & Breckenridge 1977). The system consists of up to eight fermentation vessels, each simulating the dynamics of rumen microbial metabolism. In order to inoculate the fermentation vessels with fresh rumen contents, ruminally cannulated donor animals are needed. Potential factors of variance include the ration of the donor animals, feeding intensity, time of sampling the ruminal fluid from the donor animals, chemical composition of the buffer solution and buffer flow rate through the RUSITEC fermenters. So far, no comparative studies including different laboratories have been carried out. Therefore, two ring studies with nine laboratories from Germany and Switzerland were initiated. In the first trial all groups received the same feedstuff (concentrate and hay) to be incubated under their individual conditions. Relevant information on the individual trials were documented. As expected, substantial variation in operating procedures occurred between the individual groups and several effects on the results were found. Volumes of the fermentation vessels ranged between 650 to 1100 ml. Buffer composition differed and buffer turnover ranged from 0.44 to 1/day. While pH could be kept relatively constant among groups and anaerobic conditions were maintained, the concentrations of NH_3 (10.2 mmol/l ± 2.9 SD) and SCFA (63.4 mmol/l ± 20.6 SD) varied considerably. The degradability of DM and NDF averaged 53% ± 10 SD and 41% ± 18, respectively. In the second experiment identical operating procedures have been carried out, with the donor animals as the only individual factor. Across all fermentation parameters and both feed types, the variation of the parameters in trial 2 was 14.1% lower than in trial 1. For hay, the variation in the 2nd trial was 17.7 % lower compared with trial 1. For concentrate/hay mixture, the variation in the 2nd trial was 10.3 % lower than in trial 1. The variation was lower for all degradability parameters in the 2nd trial. Across all degradability parameters and both feed types, the variation was 31.7 % lower in trial 2 than in trial 1. In conclusion, the results of all groups were in a similar range as compared with literature data. However, in order to guarantee a better degree of comparability a standardized protocol is recommended.

Reference

Czerkawski, J., Breckenridge, C., 1977. British Journal of Nutrition 38, 371-384. https://doi.org/10.1079/bjn19770102

Validation of a new estimation equation for nitrogen excretions of dairy cows with on-farm data

C. Dreyer[1], B. Losand[2], H. Spiekers[3], P. Sanftleben[1], J. Hummel[4]
[1]Institute of Livestock Farming of the Mecklenburg-Vorpommern Research Centre for Agriculture and Fisheries, Dummerstorf, Germany. [2]DLG e.V., Frankfurt am Main, Germany. [3]Institute for Animal Nutrition and Feed Management of the Bavarian State Research Center for Agriculture, Grub, Germany. [4]Department of Animal Sciences of the Georg-August-University Göttingen, Göttingen, Germany (c.dreyer@lfa.mvnet.de)

Recently, a new estimation formula for nitrogen excretions has been published for German dairy farms (Honig et al., 2024; “MoMiNE”-project). The equation has been developed using data from research stations and intends to predict nitrogen excretions solely on the basis of milk recording data. To validate the estimation equation with practical data from dairy farms, two Holstein farms in north-east Germany were monitored for 16 months. Farm A has 330 dairy cows and an automatic milking system while farm B is milking 560 dairy cows in a milking parlor. Besides the monthly milk recording analysis data and bulk tank milk analysis, also information about feed and nutrient intake in different feeding groups as well as information about disease diagnoses of both farms were available. Only considering cows in lactation, in farm A feed intake data was measured daily for 3 rations and 4 feeding groups; and in farm B it was measured weekly for 4 rations and 6 feeding groups. The ration calculations and the dates of their changes were combined with feed intake per feeding group to generate nutrient intakes. It resulted in 280 diet x feeding group combinations for farm A and 174 for farm B with 2 to 4 values per month. Documentations of mean days in milk and mean milk yield in the herd management program were used to connect the data of milk recording with the cows in the associated feeding group. Nitrogen excretions were calculated as the difference between nitrogen intake and nitrogen secreted with milk. Multiple linear regressions were conducted to compare the new estimation method with the one currently in use (Bannink & Hindle, 2003). For lactating dairy cows in the mid-lactation phase, an increase in the R^2- value from 0.041 to 0.085 was observed, along with a decrease in the root mean quare error (RMSE) from 68.4 to 66.8 when the new model was compared to the old model.

References

Bannink, A., Hindle, V. A., 2003. Prediction of N intake and N excretion by dairy cows from milk data. Report, 3, 0008567.
Honig, A. C., Spiekers, H., Baltissen, I., Bosch, A., Ettle, T., 2024. Modellierung der Stickstoffausscheidungen melkender Kühe. VDLUFA-Schriftenreihe 81, 472-479.

Funding

The project with the number 28N-2-048-03 is supported by funds of the Federal Ministry of Food and Agriculture (BMEL) based on a decision of the Parliament of the Federal Republic of Germany via the Federal Office for Agriculture and Food (BLE) under the innovation support programme.

Investigating the impact of sheep faeces preservation methods on microbial activity and gas production in an *in vitro* environment

L. Rippstein[1], J.S. Saénz[1,2], N. Titze[1,2], K. Wild[1], J. Seifert[1,2], M. Rodehutscord[1,2]
[1]Institute of Animal Science, University of Hohenheim, Stuttgart, Germany. [2]Hohenheim Center for Livestock Microbiome Research, University of Hohenheim, Stuttgart, Germany (jseifert@uni-hohenheim.de)

In vitro systems involving microbial fermentation, such as the Hohenheim gas test (HGT), typically need freshly obtained inocula such as rumen fluid or faeces. The objective of this study was to test the possibility of becoming independent of fresh animal material for *in vitro* digestibility estimation by using preserved faeces. Fresh faeces from sheep (control, C) was compared with differently preserved faeces in the HGT by using nine feeds and studying *in vitro* gas production (GP) and the active fraction of the microbiome. Preservation involved freezing at -20°C (FR), shock-freezing with liquid nitrogen (N) and additional freezing at -20°C (FRN), FRN followed by defrosting (FRNdef), shock-freezing with liquid N and freeze-drying (FDN), freeze-drying (FD), freeze-drying and storage for three weeks (FD3W) and six months (FD6M). All treatments were used for GP measurements in a HGT. Metaproteomics was used to analyse the effects of treatments on microbial community composition and function. On average of the nine feeds, the potential GP with FR and FRN (61 mL/200 mg DM) was comparable to that of C (62 mL/200 mg DM), whereas values for FRNdef, FDN, FD, FD3W, and FD6M accounted for only 85, 78, 76, 78, and 71%, respectively, of the potential GP obtained with C. The two-way interaction of feed and preservation treatment was significant ($p < 0.001$) for potential GP, GP rate, and the lag phase. The findings on GP were confirmed by metaproteome analyses. Identified peptides were clustered in 24,654 protein groups, with datasets from C, FR, and FRN differing significantly from FD and FDN due to the relative abundance of the core proteins ($p < 0.001$). A significant decline of Bacteroidota and functional redundancy values, as well as of specific proteins such as carbohydrate esterases ($p < 0.05$) and glycoside hydrolases ($p < 0.01$) were identified in FD and FDN treatments. In general, frozen faeces showed the smallest difference compared to fresh faeces and could serve as a viable alternative inoculum source to replace fresh faeces in the HGT.

Funding
This work was supported through a doctoral scholarship for L. Rippstein by the H. Wilhelm Schaumann Foundation (Hamburg, Germany).

Establishment of an in vitro model to evaluate the energy production and transport in rumen enterocytes

E. Benassai[1], L. Jing[2], N. Ali[2], E. Dupuis[1], T. Chalvon-Demersay[1], E. Coudert[1]
[1]CMI Roullier, Nutrition Animale et Environnement, Saint Malo, France. [2]CMI Roullier, Sciences Physico-chimiques et Bio-analytiques, Saint Malo, France (emilia.benassai@roullier.com)

In cattle, the rumen is a crucial organ for digesting essential macronutrients, such as carbohydrates and proteins. While the role of rumen microbiota in feed efficiency is well-documented, less is known about the rumen epithelium's role in absorbing secondary metabolites. Notably, short chain volatile fatty acids (SVCFAs) are the major energy source for ruminants, and their absorption is crucial for global homeostasis. This study aims to establish an *in vitro* functional model of rumen enterocyte SCVFA transport and energy production. We utilized the immortalized bovine rumen cell line (BREC1), provided by Prof. Honglin Jiang from Virginia Tech (Ji et al., 2021), which expresses key SCVFA transporters like monocarboxylate transporters 1 and 4 (MCT-1 and MCT-4). These cells retain significant physiological and metabolic traits of primary rumen cells. Based on previous studies on yak rumen cells (Wang et al., 2022), BREC1 cells were cultured on Transwell inserts to develop a transport model. Preliminary results indicate an increase in transepithelial electrical resistance (TEER) values when cells are cultured on collagen-IV coated inserts up to 18 days. Additionally, NH_4^+ transport from the apical to the basolateral chamber was measured using a colorimetric assay. The lower transport percentage in cell-coated inserts compared to cell-free controls indicates that a barrier was established, consistent with the ruminal wall's *in vivo* function in regulating ammonia production during bacterial protein digestion. Further assessment of actual differentiation is planned by evaluating the expression of key SCVFA transporters, MCT-1 and MCT-4, under polarized conditions and comparing it to un-polarized controls. Further experiments will investigate the effects of the three most abundant SCVFAs in rumen fluid—acetate, propionate, and butyrate—on BREC1 cell energy metabolism. Cellular bioenergetics will be assessed by measuring the oxygen consumption rate (OCR) using Resipher technology, and ATP production will be quantified using an ATP detection kit. This will allow a quantitative assessment of the energy produced by BREC1 cells under different SCVFA treatments, determining if BREC1 cells utilize SCVFAs as an energy source consistent with *in vivo* conditions. In conclusion, this work aims to establish a robust *in vitro* model to elucidate SCVFA metabolism and transport mechanisms in rumen enterocytes. Insights from these studies could ultimately contribute to optimizing bovine nutrition and enhancing feed efficiency. The results of ongoing experiments are anticipated to be shared at the conference, providing a foundation for further investigations into the regulation of nutrient absorption and energy metabolism in the bovine rumen epithelium.

References

Ji, X., Tong, H., Settlage, R., Yao, W., Jiang, H., 2021. Journal of Animal Science, 99, skab273. https://doi.org/10.1093/jas/skab273
Wang, J., Hu, R., Wang, Z., Guo, Y., Wang, S., Zou, H., Peng, Q., Jiang, Y., 2022. Oxidative Medicine and Cellular Longevity, 2022, 8128028. https://doi.org/10.1155/2022/8128028

Comparison of in vitro rumen fermentation characteristics of camelina, hempseed and rapeseed cake for supplementing dairy cow diets

M. Lamminen, I. Korhonen, T. Kokkonen, A. Vanhatalo, A. Halmemies-Beauchet-Filleau
University of Helsinki, Helsinki, Finland (marjukka.lamminen@helsinki.fi)

Camelina and hempseed cake are by-products of vegetable oil production, which have so far been scarcely used in animal nutrition. They contain high levels of high-quality protein and unsaturated fat and would thus be valuable supplementary feeds for livestock. Rapeseed is the most important protein feed for Finnish dairy cows, but challenges related to plant protection hinder its cultivation, and rapeseed yields and cultivation areas have decreased in recent years. Therefore, there is demand for extending the range of domestic protein feeds. The aim of this study was to compare the effects of camelina, hemp and rapeseed cakes on in vitro rumen fermentation characteristics. Twelve treatments consisted of combinations of four protein feeds (negative control without protein feed, camelina cake, hempseed cake or rapeseed cake) and three forages (grass, red clover or maize silages). Treatments also contained barley, and forage:concentrate ratio was 50:50 in diet dry matter (DM). Feeds were analysed for DM, crude protein (CP), total fat (excluding forages) and neutral detergent fibre (NDF). Rumen digesta was collected from two rumen-cannulated Nordic Red dairy cows. The ratio of filtered (250 µm sieve) rumen content and buffer (McDougall, 1948) was 1:2 (v:v). Rumen fermentation was determined at 1, 4, 8, 12, 24 and 30 h from the beginning of the incubation. The experiment was repeated four times with Gas Endeavour (Bioprocess control, Lund, Sweden) in vitro system. The CP concentration of negative controls ranged from 95 to 140 g/kg DM depending on the forage source. The CP concentration of protein supplemented diets was 170 g/kg DM. Camelina, hempseed and rapeseed cake contained CP 367, 286 and 332 g/kg DM, total fat 131, 44 and 131 g/kg DM, and NDF 211, 482 and 194 g/kg DM. Protein supplementation did not increase the concentration of volatile fatty acids (VFA) in rumen liquid. The total VFA concentration did not differ between rapeseed and camelina cake. Compared to rapeseed, camelina increased the concentration and molar proportion of propionate and reduced the molar proportion of butyrate. The concentration of total VFA was lower in hemp than in camelina and tended to be lower than in rapeseed. Compared to rapeseed and camelina, hemp resulted in lower concentration of propionate and higher molar proportion of acetate. The effects were not dependent on the forage species. Based on the results, the composition and in vitro rumen fermentation characteristics of camelina and rapeseed are very similar and might thus correspond to one another in dairy cow nutrition. The feed value of hempseed cake may be lower than that of camelina and rapeseed due to higher NDF concentration.

Reference

McDougall, E.I., 1948. Biochemical Journal 43, 99–109. https://doi.org/10.1042/bj0430099

Funding

Project received funding from The Finnish Association of Academic Agronomists.

Rumen microbial community and functional response to high- and low-starch diets: Metagenomic evidence from original Brown Swiss cows

M. Z. Islam[1], J. Lan[2], H. Zehnle[3], Y. Li[1], F. Wahl[4], E. Slack[2], M. Schoelmerich[3], R. Zenobi[5], S. Giannoukos[5], S. E. Räisänen[1,6], M. Niu[1]
[1]Animal Nutrition, Department of Environmental Systems Science, ETH Zürich, 8092 Zürich, Switzerland. [2]Department of Health Sciences and Technology, ETH Zürich, 8093 Zürich, Switzerland. [3]Environmental Microbiology, Department of Environmental Systems Science, ETH Zürich, 8092 Zürich, Switzerland. [4]Food Microbial Systems Research Division, Agroscope, 3003 Bern, Switzerland. [5]Department of Chemistry and Applied Biosciences, ETH Zürich, 8093 Zürich, Switzerland. [6]Department of Animal and Veterinary Sciences, Aarhus University, 8830 Tjele, Denmark (mutian.niu@usys.ethz.ch)

This study aimed to explore how dietary starch levels influence rumen microbial communities and their metabolic functions using a metagenomic approach, as part of a broader multi-omics integration project. A switchback design was employed over three 9-day periods (7 days of adaptation followed by 2 days of sampling) using four rumen-cannulated Original Brown Swiss cows. Animals were alternately fed low-starch (LS; 6.31% DM) and high-starch (HS; 16.2% DM) total mixed rations in an ABA/BAB sequence. Rumen contents (both solid and fluid fraction) were sampled 2 h post-feeding and subjected to shotgun metagenomic sequencing. Taxonomic composition was estimated directly from metagenomic raw reads using SingleM, while the differential abundance of bacterial and archaeal taxa was evaluated via MetaCoder, with statistical comparisons conducted using the Wilcoxon rank sum test (adjusted $P \leq 0.10$). For functional profiling, metagenomic assemblies (>1000 base pairs) were processed with Prodigal to predict open reading frames, and annotations were assigned via Hidden Markov Model searches against the KOfam database. The KEGG orthologs were further categorized into 11 major metabolic functions, and feature-level differential abundance was assessed using MetagenomeSeq (adjusted $P \leq 0.10$; log2 fold-change with cumulative-sum scaling). Functional pathway enrichment analysis was conducted with reference to the KEGG database. Taxonomic profiling indicated a significant dominance of *Bacteroidia* in the LS diet and *Clostridia* in the HS diet ($P < 0.05$), consistent with known fiber- versus starch-degrading microbial preferences. Functional annotation revealed similar overall distributions of carbohydrate and glycan biosynthesis pathways in both diet groups. Although no individual KEGG orthologs showed significant differential abundance, pathway enrichment analysis highlighted consistent enrichment ($P < 0.05$) of carbohydrate-related metabolic routes, including glycolysis, pyruvate metabolism, galactose metabolism, and starch and sucrose metabolism across both dietary treatments. In summary, dietary starch levels induced clear shifts in the structure of the rumen microbial community, particularly in taxa associated with fiber and starch metabolism. However, the metagenomic functional profiles based on predicted gene content remained largely unchanged between diets. This suggests that while the microbial composition adapts to dietary changes, the overall functional potential encoded in the metagenome is relatively stable in the short term. Ongoing metatranscriptomic analyses will help clarify whether these structural changes are accompanied by shifts in gene expression and active microbial metabolism under varying starch inputs.

Peripheral blood mononuclear cells as bioenergetic biomarkers for metabolic stress in pigs

L. Cavaglieri, G. Rimbach, I. Ipharraguerre
Institute of Human Nutrition and Food Science, University of Kiel, Kiel, Germany
(ipharraguerre@foodsci.uni-kiel.de)

Over recent decades, metabolic disorders in farm animals have risen, particularly among those genetically selected for traits such as rapid growth and high milk yield. According to life history theory, selection pressure may have compromised their ability to allocate energy efficiently, reducing adaptive capacity and increasing susceptibility to metabolic stress (Rauw, 2012). Mitochondria play a central role on cellular metabolism, and their dysfunction is linked to metabolic disorders, making the analysis of mitochondrial bioenergetics a valuable tool for evaluating metabolic health. Circulating cells such as peripheral blood mononuclear cells (PBMCs) with functional mitochondria respond to peripheral metabolic and inflammatory stressors, positioning them as key biomarkers of systemic mitochondrial function (Wilkinson and Dunham-Snary, 2023). PBMCs offer valuable insight into metabolic and immune stress, making them an ideal model for bioenergetic studies in livestock. However, their potential remains underexplored, limiting their use in translational research. This study aimed to characterize the bioenergetic responses of growing pigs' PBMCs and establish a standardized protocol for analysis. PBMCs were isolated from six healthy, three-month-old pigs using density gradient separation and incubated at two densities (1.5×10^5 and 2.0×10^5 cells/well). Cells were incubated either resting (control) or activated with various concentrations and combinations of phorbol 12-myristate 13-acetate (PMA; 20, 40, and 200 ng/mL), ionomycin (ION; 8 and 10 µg/mL), and bacterial lipopolysaccharide (10 and 25 µg/mL). The Seahorse XF Extracellular Flux Analyzer (Agilent, Seahorse XFe96 Analyzer) was used to perform mitochondrial stress tests and thereby obtain key bioenergetic parameters, including ATP-coupled respiration, basal and maximal respiration, and spare respiratory capacity (SRC). Results were analyzed using a generalized linear mixed model (PROC GLIMMIX, SAS 9.4) to evaluate the effects of activation protocol and cell concentration on the aforementioned parameters, with pigs included as a random effect. PBMCs seeded at 1.5×10^5 cells/well did not exhibit significantly higher metabolic activity compared to those at 2.0×10^5 cells/well ($P > 0.05$), with no differences observed in basal respiration, ATP- coupled respiration, maximal respiration, or SRC. Among the tested immunogens, all concentrations of PMA combined with ION at 8 µg/mL induced the highest metabolic activation, significantly surpassing ($P <$ .0001) the unstimulated control. These findings highlight the potential of PBMCs as a bioenergetic model for pigs, enabling research into immune-metabolic interactions relevant to animal health. Establishing a standardized methodology for PBMC bioenergetic assessment enhances translational research by facilitating early detection of metabolic stress and immune activation. While this study provides a foundation, further research should explore the effects of genetic background, diet, sex, and physiological status to refine the model. Advancing our understanding of PBMC bioenergetics in livestock could improve metabolic health monitoring and support the development of nutritional and management strategies that optimize immune function and productivity.

References

Rauw, W.M., 2012. Frontiers in Genetics 3, 1-14. https://doi.org/10.3389/fgene.2012.00267
Wilkinson, M.S., Dunham-Snary, K.J., 2023. Trends in Endocrinology and Metabolism 34, 554-570. https://doi.org/10.1016/j.tem.2023.06.004

In vivo protein synthesis response of porcine peripheral blood mononuclear cells to lipopolysaccharides and deoxynivalenol

S. Dänicke[1], E. Bannert[1], T. Tesch[1], S. Kersten[1], S. Bühler[1], S. Kahlert[2], H.-J. Rothkötter[2], J. Kluess[1], J. Frahm[1]
[1]FLI Braunschweig, Braunschweig, Germany. [2]Otto-von-Guericke-Universitaet Magdeburg, Magdeburg, Germany (sven.daenicke@fli.de)

Parenteral application of LPS is commonly used to study systemic inflammation (SI) in animals. Peripheral blood mononuclear cells (PBMCs) are closely involved in triggering and resolution of SI. It is unclear however, whether the route of LPS entry to the systemic circulation further modifies these processes. Using similar lipopolysaccharides (LPS) doses, the load of LPS to PBMC might be different when derived from the gastrointestinal tract (pre-hepatic) compared to the systemic circulation (post-hepatic). Orally ingested deoxynivalenol (DON) is rapidly absorbed in pigs and has the potential of inhibiting protein synthesis. Less is known about the effects of DON on the protein synthesis of PBMC in the early stage of SI when induced either pre- or post-hepatically in pigs. Thus, six groups of pigs were tested; three groups were fed a control diet while the other three were orally exposed to DON (C and D, first letter of group designation, respectively) for four weeks prior to induction of SI *via* the pre- and post-hepatic LPS (L, third and second letter of group designation, respectively) entry route in comparison to saline administered corresponding controls (C) (CCC, CCL, CLC and DCC, DCL, DLC, respectively). Full details of the experiment were described earlier (Bannert et al., 2015; Dänicke et al., 2020). L-Phenylalanine-d5 was administered intravenously 105 min after LPS or saline treatment for isotope tracing. PBMC were isolated from jugular blood sampled sequentially until 90 min post d5-phe and measured for isotopic enrichment as described previously (Dänicke et al, 2020). Proteins of PBMCs that were newly synthesized amounted to 13.0±1.2, 8.7±1.2, 8.2±0.9, 14.4±0.9, 10.0±1.1 and 10.4±1.9 percent of total proteins present in PBMCs in groups CCC, CCL, CLC, DCC, DCL and DLC, respectively. When compared to their feeding controls, LPS administrations reduced protein synthesis by 35% and 29% in control and DON fed groups, respectively, irrespective of LPS entry route. However, only the differences between CCL/CLC and DCC were proved to be significant ($p<0.05$). The LPS-induced drop in PBMC's total protein synthesis rate in the examined early phase of SI coincided with a reduced arterial O_2-pressure, a pronounced lactic acidosis (Bannert et al., 2015) and a reduction of hepatic metabolic function (Dänicke et al, 2020) hinting at metabolic and energetic stress for PBMC. The pronounced increase in inflammatory cytokines observed in the same time period (Tesch et al., 2016) further suggests that PBMC either prioritized synthesis of proteins situationally necessary, secreted pre-formed cytokines or that peripheral cytokine levels originated from other sources, e.g. from liver. Whether the suggested processes occurred together causing the overall effect on protein synthesis reduction could further be studied by a differential evaluation of cell types constituting to the PBMC fraction and by studying genes directly involved in regulation of protein synthesis. The co-exposure to DON failed to modify the observed LPS-effects.

References

Bannert, E., Tesch, T., Kluess, J., Frahm, J., Kersten, S., Kahlert, S., Renner, L., Rothkötter, H.J., Dänicke, S., 2015. Toxins 7, 4773-4796. https://doi.org/10.3390/toxins7114773
Dänicke, S., Bannert, E., Tesch, T., Kersten, S., Frahm, J., Bühler, S., Sauerwein, H., Görs, S., Kahlert, S., Rothkötter, H.-J., Metges, C.C., Kluess, J., 2020. Innate Immunity 26, 1753425920937778. https://doi.org/10.1177/1753425920937778
Tesch, T., Bannert, E., Kluess, J., Frahm, J., Kersten, S., Breves, G., Renner, L., Kahlert, S., Rothkötter, H.J., Dänicke, S., 2016. Toxins 8, 3. https://doi.org/10.3390/toxins8010003

A non-invasive test to determine oro-caecal transit time in breath and saliva in immunostimulated pigs

M. Cavalleri[1], S. Görs[1], A. Vernunft[1], H. Reyer[1], J. Kluess[2], S. Dänicke[2], J. Zentek[3], K. Wimmers[1,4], C.C. Metges[1,4], Q.L. Sciascia[1]
[1]Research Institute for Farm Animal Biology (FBN), Dummerstorf, Germany. [2]Federal Research Institute for Animal Health, Braunschweig, Germany. [3]Freie Universität Berlin, Veterinary Faculty, Berlin, Germany. [4]University of Rostock, Faculty of Agricultural and Environmental Sciences, Rostock, Germany (metges@fbn-dummerstorf.de)

To study intestinal transit in vivo in pigs, intestinal cannulas are implanted to gain access to intestinal contents, which is a highly invasive procedure. An alternative, non-invasive method is to use orally administered tracer substances that reflect the time-dependent metabolism in the intestine. In humans, lactose-^{13}C-ureide (L^{13}CU), which cannot be degraded in the small intestine, has been used to measure the transit time through the upper intestinal tract. The time at which L^{13}CU is oxidized by the caecal microbiota can be determined by measuring the ^{13}C enrichment of CO_2 in the breath (oro-caecal transit time, OCTT). It is unknown whether OCTT can be measured in the breath and saliva of pigs and whether fiber and immune stimulation affect it. To this end, we determined the OCTT with the L^{13}CU test in breath and saliva CO_2 samples when male Landrace pigs were fed a low (n=22) or high (n=22) fiber diet (LF, 2.8 % fiber; HF 6.5 % fiber) and after an i.m. injection treatment (Trt) with either lipopolysaccharide (LPS) or NaCl in a 2 x 2 (fiber/trt) factorial design. The HF and LF diets were fed isoenergetically from age d 53. On age day (d) 70, vein catheters were implanted for red blood cell collection, and on d 75, 24 hours (h) before L^{13}CU administration, half of the pigs per diet group received an i.m. LPS or saline injection, respectively. Twelve h after the injection, a portion of feed was spiked with a 0.4 g priming dose of unlabeled lactose-ureide, and 12 h after an overnight fast, the pigs were given a feed portion containing 0.4 g L^{13}CU powder (99 atom% ^{13}C), followed by one-third of the daily feed allowance. Saliva and breath samples were collected before (basal) and 1, 1.5, 2, 2.5, 3, 3.5, 4 h and hourly until 12 h and again 24 h after L^{13}CU-administration. The OCTT was derived from the time at which ^{13}C enrichment of CO_2 in breath and saliva, measured by isotope ratio mass spectrometry, was significantly higher (+2 $\Delta\delta^{13}$C, ‰) than the pre-tracer baseline. Administration of LPS 24 h before the L^{13}CU challenge resulted in a delayed increase in $^{13}CO_2$ accumulation in the breath of LF pigs, which was associated with a prolonged OCTT compared to LF NaCl and HF LPS pigs (3.4 vs. 2.9 h; $p < 0.05$). The lack of an effect of LPS on OCTT in HF-fed pigs (3.1 h) is likely a result of the higher production of SCFAs, which attenuates the inflammatory response associated with decreased intestinal smooth muscle contractility. OCTT estimates in saliva CO_2 were consistent with those in breath, and we conclude that saliva is generally suitable as a sample matrix for OCTT, but its use requires further validation.

Funding
This work is an outcome of the PIGWEB project funded by the European Union’s Horizon 2020 research and innovation program under Grant Agreement No 101004770.

GenoPHEnix – a European research infrastructure for genome - phenome interactions in sustainable animal production

R. Baumont[1], K. Wimmers[2], M. Boichard[3], E. Clark[4], T. Hartwig[2], E. Labussière[5], C. Larzul[6]
[1]INRAE, UMR Herbivores, Theix, 63122 Saint-Genès-Champanelle, France. [2]Research Institute for Farm Animal Biology (FBN), 18196 Dummerstorf, Germany. [3]INRAE, GABI, 78352 Jouy-en-Josas, France. [4]EMBL-EBI, Wellcome Genome Campus, Hinxton CB10 1SD, United Kingdom. [5]INRAE, PEGASE, 35590 Saint-Gilles, France. [6]INRAE, GenPhySE, 31320 Castanet-Tolosan, France (etienne.labussiere@inrae.fr)

The European Strategy Forum on Research Infrastructures Landscape analysis identified a critical gap for farm animal genotyping, phenotyping, conservation of genetic resources, and breeding necessitating an integrated pan-European approach. With this specific gap in mind for the creation of a research infrastructure that would provide a long-term, fully collaborative and coordinated institutional framework to the scientific community, the European farm animal genomics and phenotyping research community has resolved to apply for the creation of a new research infrastructure: GenoPHEnix. GenoPHEnix aims to be a pan-European, multi-species, multidisciplinary platform for animal genetic and multiomics resources, phenotyping, genome analyses, and sustainable breeding and management, focused on improving animal welfare, health, resilience, and efficiency. GenoPHEnix builds on 1) the international initiative FAANG (Functional Annotation of Animal Genomes) and the INFRADEV EuroFAANG RI concept development project that works toward the development of the Genome to Phenome approach (G2P), and by 2) the INFRAIA projects AQUAEXCEL 3.0, PIGWEB and SmartCow that have been instrumental in advancing efficient and ethical species-specific phenotyping methods for farmed animals, and in offering transnational access to high-level experimental facilities for the academic and industry research communities. GenoPHEnix will support cutting-edge research in animal science while developing and delivering methods, technologies and innovative approaches to solve the challenges to transform the livestock and aquaculture production systems within the context of One Health and European Competitiveness. GenoPHEnix is designed 1) to sustainably produce and manage healthier farmed animals, with the highest welfare standards; 2) to more accurately exploit animal variability through enhanced phenotyping and genotyping capacity; 3) to contribute to the 3Rs (reduction, refinement, replacement) in animal research by providing suitable models for deep in vitro as well as in vivo phenotyping; 4) to advance the analysis of genome function, combined with the collection of phenotypes and multiomics information at cell, tissue, animal and on-farm/population level resolution; and 5) to provide tools and knowledge for optimal and more precise breeding and animal health considering sustainable management practices, and conservation of genetic diversity. GenoPHEnix will improve the leadership and excellence of European farm animal research community by 1) sharing experimental capacity and providing access for genotyping and non-and minimally invasive large-scale phenotyping of farmed animals in complementary environmental conditions; 2) expanding biobanking capabilities and access to animal genetic resources and cellular models; 3) promoting standardisation and FAIRisation of animal genome and phenome data; 4) seeking synergies with existing projects and other RIs to consolidate European farm animal research in the agri-food domain; 5) training to promote the best practices in farm animal science. GenoPHEnix unites scientific partners from 11 countries, is supported by the European Forum of Farm Animal Breeders and aims, while established, at integrating new partners.

Validation of the ZELP Sense™ device for measuring methane and carbon dioxide emissions in cattle

M. Battelli[1], S. Colombini[1], M. Battini[1], R. Bica[2], S. Silvestri[2], L. Rapetti[1]
[1]Department of Agricultural and Environmental Sciences, University of Milan, Milan, Italy. [2] ZELP, London, United Kingdom (marco.battelli@unimi.it)

Ruminants are major contributors to agricultural greenhouse gas emissions, mainly due to methane (CH_4) produced from rumen fermentation. Accurately measuring CH_4 emissions is crucial for assessing ruminant farming's environmental impact and evaluating CH_4 reduction strategies. Several techniques exist for measuring individual CH_4 emissions, with the most used being respiration chambers (RC), the sulphur hexafluoride (SF_6) tracer method, and the GreenFeed automated system. These techniques vary in key aspects, such as sampling method (spot or continuous), experiment duration, number of animals required or allowed, and required conditions, which may affect behaviour. Additionally, cost is a key constraint, encompassing both equipment expenses and the overall execution of the study. In this context, ZELP is developing an innovative device called Sense, designed to monitor individual CH_4 and carbon dioxide (CO_2) emissions in cattle. The Sense device is designed to enable continuous, high-frequency analysis of gases from breath. A lightweight device, made from ventilated neoprene, is fitted to the cow's muzzle and can be used in any context, including in barn and on pasture. Respiration rate from onboard sensors, personalised tidal volume estimates, and measured dilution coefficients are combined with gas concentration measurements to calculate daily CH_4 and CO_2 emissions. Data can be recorded continuously or intermittently and stored or transmitted wirelessly. This trial aims to assess the Sense device's impact on animal behaviour and validate its accuracy in measuring gas production. To achieve this, emissions data (or gas production data) from the Sense device will be directly compared to simultaneous measurements from individual open-circuit respiration chambers, the gold standard for such studies.The trial will involve four multiparous, non-lactating cows housed in a free-stall barn. Before the trial, cows will be adapted to the Sense device and to the respiration chambers in isolation. Cows will be video recorded before and during the adaption period to collect changes in behaviour and signs of discomfort (if any) due to the Sense device. During the trial, each cow will spend four separate 4-day periods in the respiration chamber with the Sense device on, returning to the free-stall barn after each period. Each cow will be assigned a specific Sense device for the entire trial, while the cow-to-chamber assignment will rotate in each period to account for potential biases.Behavioural data will be analysed with BORIS software and compared using repeated measures ANOVA. Gas production (or emissions) data (CO2 and CH4) from the Sense device and respiration chambers will be compared using a Bland-Altman plot and concordance correlation coefficient (CCC).

The relationship between metabolic gas emissions and performance in Angus bulls and heifers

M.A. Harrison, S. Reilly, K. Carmichael, L. Cavani, S. Zimmerman
C-Lock Inc., Rapid City, USA (mharrison@c-lockinc.com)

Methane (CH_4) production is a moderately heritable trait. However, selection based on methane production may reduce economically relevant traits, which decreases CH_4intensity (g of CH_4 per kg of ADG) and overall profitability. The objective of this project was to evaluate the relationship between gas emissions and animal performance characteristics. Individual animal feed intake and body weights (BW) were recorded daily using SmartFeed and SmartScale (C-Lock Inc., USA) from 181 Angus bulls and 113 Angus heifers during a 64-d and 60-d performance test, respectively. Performance traits were composed of metabolic BW, average daily gain (ADG), dry matter intake (DMI), and residual feed intake (RFI). During the test period, 2 GreenFeeds (GF; C-Lock Inc.) were used to collect metabolic gas fluxes. Animals were rotated every 8 to 21 d through pens with the GF, so all animals were exposed to GF during the performance test. Only animals with at least 20 GF visits were included in the analysis, resulting from data from 127 bulls and 80 heifers. Daily averages (g/d) of CH_4, carbon dioxide (CO_2), and oxygen (O_2) were 167 ± 26.7, 7111 ± 638, and 5488 ± 483 for bulls; and 179 ± 22.7, 6681 ± 673, and4282 ± 478 for heifers. In addition to CH_4 production, CH_4 intensity, CH_4 yield (g of CH_4 per kg of DMI), and residual methane emissions (RME) were evaluated. RME was computed as the residuals from a regression of CH_4 production on DMI and metabolic BW. Individual metabolic heat production (HP; kcal) was also calculated using the Brouwer Equation. Average CH_4 intensity, CH_4 yield, and RME (g/d) were 110 ± 42, 21.1± 4.4, and 0 ± 26.6 for bulls; and 202 ± 1754, 17 ± 5.7, and 0 ± 18.1 for heifers. Gas fluxes were correlated with greater metabolic BW (from 0.10 to 0.63), ADG (from 0.03 to 0.61), DMI (from 0.07 to 0.60), and RFI (from 0.07 to 0.32). In bulls, CH_4 intensity was negatively correlated with BW (-0.35), ADG (-0.79) and DMI (-0.35), but not correlated with RFI. In both bulls and heifers, CH_4 yield was negatively correlated with BW (-0.28 and -0.39), ADG (-0.33 and -0.25), DMI (-0.61 and-0.92), and RFI (-0.47 and -0.83). RME was favorably correlated with RFI in heifers (0.41). For bulls and heifers, HP was positively correlated with BW (0.60 and 0.42), ADG (0.57 and 0.28), DMI (0.50 and 0.49), and RFI (0.13 and 0.28). The findings of this study suggest that selection for methane reduction may favor feed efficiency in Angus cattle.

Different models to estimate the net energy of finishing diets for *Bos indicus* using gas flux from the automated head chamber

H.M. Alhadas[1], J.T Silva[2], A.K. Schilling-Hazlett[2], L.M. Campos[2], K.R. Stackhouse-Lawson[2], S.E. Place[2], J.J. Vargas[2], P.H.V. Carvalho[2]
[1]Federal University of Viçosa, Viçosa, Brazil. [2]Colorado State University, Fort Collins, USA (herlon.alhadas@ufv.br)

The objective of this work was to evaluate different approaches for estimating heat production (HP) and the net energy (NE) of diet during the finishing period, using gas flux from an automated head chamber system (AHCS; GreenFeed, C-Lock, Rapid City, SD, USA). One hundred and thirteen Brahman steers [body weight (BW) = 438 ± 38] fed a similar diet for 96 days were used. Steers were allocated in two pens containing one AHCS to measure CO_2 and CH_4 production and O_2 consumption. The initial and final weights were measured on d0 and d96. Using gas flux, two models were used to estimate HP: 1) ($3.866\times O_2 + 1.200\times CO_2 - 0.518\times CH_4 - 1.431\times$Urinary Nitrogen)/1.000 (Brouwer equation), and 2) 4.96+(16.07/Respiratory Quotient)×CO_2 (Kaufmann equation). However, model 1 was used without urine data. Dietary metabolizable energy (ME) was calculated as the sum of HP and retained energy (RE). Two models were used to calculate the RE: 3) $0.0635\times \text{EQEBW}^{0.75}\times \text{EBG}^{1.097}$ (EQEBW = equivalent empty BW, EBG = empty body gain; North American model, NRC); 4) $0.061\times \text{EQEBW}^{0.75}\times \text{EBG}^{1.035}$ (Brazilian model; BR-CORTE). Using the estimated ME, two cubic models were used to estimate the diet NE for maintenance and gain. These values were considered the NE gas-adjusted (gaNE), which were compared with the NE performance-adjusted (paNE), based on the equations proposed by Zinn et al. (2008). The energy requirements required to estimate paNE were calculated using Zinn et al. (2008) and BR-CORTE equations and was considered the standard values. The gaNE values obtained with the NRC were compared with those obtained by the Zinn et al. (2008) equations and the gaNE models estimated with the BR-CORTE were contrasted with paNE values from the Brazilian system. The data were analyzed in the statistical software R (v.4.4.2), considering the animal as the experimental unit and the methods as fixed effect. The Tukey test was used to compare the means. Lin's concordance correlation coefficient (CCC), Pearson correlation (r), and the significance of the slope and intercept of the regression between the centered mean and the residuals of the gaNE and paNE methods were used to compare the fit of the models. A slightly greater accuracy and precision for estimating the gaNE of the diets were obtained with the BR-CORTE equations (CCC = 0.57 vs CCC = 0.55). Furthermore, the use of BR-CORTE provided non-significant regression intercept and slope between gaNE and paNE ($P = 0.83$). However, both gaNE estimated by the Brouwer and Kaufmann equations underestimated paNE values ($P < 0.001$), although they did not differ from each other. Therefore, BR-CORTE equations provide a better fit for estimating gaNE of feedlot diets, and both HP models can be used, even without urine data for *Bos indicus* cattle.

Reference

Zinn, R.A., Barreras, A., Owens, F.N., Plascencia, A., 2008. Journal of Animal Science 86, 2680-2689. https://doi.org/10.2527/jas.2007-0561

Evaluation of the ^{13}C-bicarbonate method for determination of energy expenditure in sows: Effect of diet and ambient temperature on the respiratory quotient and ^{13}C recovery factor

A.V. Strathe[1], S. de Vries[2], M.J.W. Heetkamp[3], C. Marcussen[4]
[1]Department of Veterinary and Animal Sciences, University of Copenhagen, 1870 Frederiksberg, Denmark.
[2]Animal Nutrition Group, Wageningen University & Research, 6700AH Wageningen, Netherlands.
[3]Research Facility Carus, Wageningen University & Research, 6700AH Wageningen, Netherlands.
[4]Department of Veterinary Clinical Sciences, University of Copenhagen, 1870 Frederiksberg, Denmark (cala@sund.ku.dk)

The ^{13}C-bicarbonate method has shown to be a promising tool for short-term measurements of CO_2 production and energy expenditure in various animal species, when applied under controlled, standardized conditions. However, for reliable application, accurate estimates of the respiratory quotient (RQ), the ratio of CO_2 produced to O_2 consumed, and the ^{13}C recovery factor (RF), which reflects ^{13}C recovery efficiency, are essential. This study aimed to assess the ^{13}C-bicarbonate method against the gold standard indirect calorimetry method in sows, evaluating the effects of dietary treatments and ambient temperature on RQ and RF. Six sows (Landrace x Yorkshire, mean body weight (BW) = 268 ± 35 kg) were housed in pairs and randomly assigned to one of three treatments in a crossover design, with two sows per treatment. The treatments were: 1) a standard diet (13.5% crude protein and 0.75% lysine; 2.5 kg/day) at +25°C ($STD_{+25°C}$), 2) standard diet (2.5 kg/day) at +5°C ($STD_{+5°C}$), and 3) standard diet (2.5 kg/day) supplemented with silage (500 g/day) at +25°C ($STD+SIL_{+25°C}$). After a 5-day dietary adaptation period at 25°C, 24-hour respiratory gas exchange measurements were conducted individually in a respiration chamber at the assigned temperature, following Heetkamp *et al*. (2015). Simultaneous measurements using indirect calorimetry and the ^{13}C-bicarbonate method were performed over an 8-hour period to estimate the RQ and RF. The RF was calculated from CO_2 production measured by indirect calorimetry and ^{13}C-enrichment in the respiratory gas following an oral dose of 3 mg/kg BW of ^{13}C-labelled sodium bicarbonate given at feeding. Data from both methods were used to estimate and compare CO_2 production and energy expenditure. Analyses were performed using the MIXED procedure in SAS® (SAS Institute Inc., SAS® 9.4, 2013). There were no effects of treatments on RQ ($P > 0.05$). The least square means ± standard error for RQ were as follows: $STD_{+25°C}$ = 0.980 ± 0.029, $STD_{+5°C}$ = 0.995 ± 0.029, and $STD+SIL_{+25°C}$ = 1.023 ± 0.029. Feeding the $STD_{+5°C}$ resulted in lower ^{13}C recovery values (RF = 0.643 ± 0.05) compared to the $STD_{+25°C}$ treatment (RF = 0.855 ± 0.05; $P = 0.004$). The $STD+SIL_{+25°C}$ treatment resulted in an RF of 0.768 ± 0.05, which was not significantly different from $STD_{+25°C}$ ($P > 0.05$) but tended to differ from $STD_{+5°C}$ ($P = 0.055$). Calculations of CO_2 production and energy expenditure based on RQ and RF estimates showed no significant difference ($P > 0.05$) between the indirect calorimetry method (23.0 ± 1.3 L/kg $BW^{0.75}$/d, 493 ± 23.9 kJ/kg $BW^{0.75}$/d) and the ^{13}C-bicarbonate method (23.2 ± 1.3 L/kg $BW^{0.75}$/d, 499 ± 23.9 kJ/kg $BW^{0.75}$/d). These preliminary findings suggest that the ^{13}C-bicarbonate method can be used to estimate energy expenditure in sows.

Reference

Heetkamp, M.J.W., Alferink, S.J.J., Zandstra, T., Hendriks, P., Van den Brand, H. and W.J.J. Gerrits. 2015. Design of climate respiration chambers, adjustable to the metabolic mass of subjects. In: Gerrits, W.J.J. and E. Labussière (Eds.), *Indirect Calorimetry*, Wageningen Academic Publishers (2015), pp. 35-56

Funding

PigWeb project EU Horizon 2020

Repeatability of enteric methane emissions measurements over the production cycle of beef heifers

K.R. Martin, M. McLennan, K.M. Wood
University of Guelph, Guelph, Canada (kwood@uoguelph.ca)

Improving environmental impact of beef cattle production is an important aspect of sustainability for the beef production system. Although a plethora of research is being conducted to help reduce enteric methane emissions, often emissions for cattle are reported only over a single timeframe, and therefore it is not well established how individual animal enteric emissions can change over time and/or over different stages of the production cycle. Therefore, the objective of this study was to evaluate individual enteric methane emissions re-rankings over a three-year production cycle. A cohort of Angus crossbred heifers was monitored over four measurement periods: P1, where 72 pregnant heifers were measured in late gestation (8 weeks pre-partum); P2, where 69 animals from P1 were measured during mid-lactation; P3, where 72 animals from P1 were measured after weaning their 1st calf; and P4 where 36 heifers from P1 were measured after weaning their 2nd calf, approximately one year after P3. Animals were fed a forage-based ration *ad libitum* throughout each measurement period, with individual feed intakes measured using Insentec feeders. Enteric methane measurements were measured using C-Lock Greenfeed trailers throughout each measurement period. Overall, methane emissions (g, day $^{-1}$) found that only 5 cow's enteric methane emissions changed > |0.5| standard deviations (STD) from P1 to P2 and P1 to P3; while 4 cows changed > |0.5| STD from P1 to P4, and 6 cows changed > |0.5| STD from P2 to P3, only one cow changed > |0.5| STD from P2 to P4, and 4 cows changed > |0.5| STD from P3 to P4. Spearman rank correlations were also conducted to determine degree of re-ranking. Heifers from P1 to P2, P2 to P3, and P2 to P4 showed moderate correlations (0.49, 0.46, and 0.58, respectively; $P<0.001$), while P1 to P3, P3 to P4 (0.38, 0.45; respectively; $P<0.01$) and P1 to P4 (0.38, $P<0.05$) showed lower, but still significant correlations. This indicates that enteric methane emissions were moderately consistent from heifers to mature cows as there were limited changes in actual methane production values (g, day^{-1}) and significant Spearman rank correlations across periods. This suggests that enteric emissions measurements as measured by Greenfeeder may be a phenotypic trait which has repeatability over the production cycle for beef cattle.

Funding

This project was supported by Canadian Cattle Association, Beef Farmers of Ontario, Food From Thought: a Canada First Research Excellence Fund, and the Ontario Ministry of Agriculture and Food

Amino acid profiles in the saliva and plasma of pigs fed high and low fibre diets 6 hours after a lipopolysaccharide challenge.

Q.L. Sciascia[1], M. Cavalleri[1], S. Görs[1], H. Reyer[1], K. Wimmers[1], J. Klüß[2], S. Kersten[2], S. Dänicke[2], C.C. Metges[1]
[1]Research Institute for Farm Animal Biology (FBN), Dummerstorf, Germany. [2]Federal Research Institute for Animal Health, Braunschweig, Germany (metges@fbn-dummerstorf.de)

Immunological stress is associated with increased plasma pro-inflammatory cytokines, and changes in amino acid (AA) concentrations. Dietary fibre (DF) reduces pro-inflammatory plasma cytokine levels; the effect on AA concentrations are less well known. Amino acid concentrations are typically measured in plasma samples, yet saliva, may be a minimally invasive alternative. Therefore, the objective of this study was to assess changes in plasma cytokine levels and plasma and saliva free AA, amino-metabolite (AM) and AA-group (AG) concentrations, in pigs fed high (HF, 6.5% DF) or low (LF, 2.8% DF) fibre diets, during a lipopolysaccharide (LPS) challenge, and assess saliva as a minimally invasive alternative to plasma. At 53 days (d) of age, male littermate pairs were iso-energetically fed HF or LF diets. At 70 d, jugular catheters were fitted. At 74 d, pigs were moved to metabolic cages, and at 75 d, injected with 30 µg/kg bodyweight LPS (HF-LPS/LF-LPS) or NaCl (HF-NaCl/LF-NaCl). Rectal temperature (RT) was recorded and blood and saliva samples were collected pre- and post-LPS. Blood was sampled from the catheter and saliva collected from saliva swabs. Plasma cytokines TNF-α, IL-1β, IL-6 and IL-10 and plasma and saliva free AA, AM and AG were determined. Data were analyzed using the ANOVA or GLIMMIX procedures of SAS, corr-test function in R or Metaboanalyst. Higher RT and plasma concentrations of TNF-α and IL-6 were observed in LPS than NaCl ($P<0.01$), IL-10 and IL-1β were higher in HF-LPS than HF-NaCl ($P<0.01$), and IL-1β was lower in HF-NaCl than LF-NaCl pigs ($P=0.04$). Of the 37 saliva and plasma AA, AM and AG, the concentration of 30 were higher and 4 were lower in plasma than saliva, whilst 3 were not different between the two matrices. At 6 h post-LPS, the concentration of 28 were higher and 3 were lower in plasma than saliva, and there was no difference in the concentrations of 6 between the two matrices. Spearman correlation showed that only taurine (HF-NaCl, $R=0.905$ $P=0.005$), tyrosine (LF-NaCl, $R=0.857$, $P=0.01$) and serine (LF-LPS, $R=0.786$, $P=0.03$) concentrations correlated between saliva and plasma. Partial-least squares discriminate analysis identified that plasma γ-aminobutyric acid, alanine and aspartate contributed to HF-LPS vs HF-NaCl, whilst taurine, α-aminoadipic acid, γ-aminobutyric acid, tryptophan, isoleucine, alanine, β-alanine and tyrosine contributed to the LF-LPS vs LF-NaCl group separation. These results show that increased DF did not affect RT or plasma pro-inflammatory cytokine concentrations in response to an LPS challenge. With the exception of hydroxyproline, all AA and AM could be quantified in both saliva and plasma, with the majority having a lower concentration in saliva than plasma. Lastly, there was only a low correlation between plasma and saliva whilst potential biomarkers of an LPS-challenge could only be identified in plasma.

Funding

This work is an outcome of the PIGWEB project and has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 101004770.

Evaluate the body composition of growing pigs fed with two SID lysine levels using computed tomography

X. Guan, J. Fledderus, M. de Mik, F. Molist
Schothorst Feed Research, Lelystad, Netherlands (xguan@schothorst.nl)

Body composition of pigs can be measured by indirect calorimetry or the slaughter method. Compared to the slaughter method, which measured different pigs at each time point, computed tomography (CT) enabled repeated measurement in the same pig over time. The objective of the present study was to measure the body composition of growing pigs fed with two different SID lysine levels. At approximately 75.3 kg body weight (BW) and age of 17-weeks, 28 female pigs (TN70 X Tempo) entered the study and were scanned by CT under anaesthesia to obtain the body composition. Pigs were randomly allocated to one of two isoenergetic diets providing 9.65 MJ net energy (NE) per kg of feed. Treatment 1 contained 13.4% crude protein (CP) and 7.5 g SID lysine per kg of feed, and treatment 2 contained 12.9% CP and 5.25 g SID lysine per kg of feed. Pigs followed a semi-ad libitum schema under a NE intake at 3.2 x maintenance requirements based on metabolic BW. At approximately 110 kg BW, pigs were weighed and scanned again by CT. Pixel data from CT corresponding to fat and muscle component in the body mass were converted to absolute weight. The data was analysed by ANOVA with Genstat®. Treatment means were compared by least significant difference (LSD) after significant effects were confirmed by ANOVA. Statistical significance was defined as $P < 0.05$. The starting BW of pigs in treatment 1 and 2 were 75.7 ± 5.48 kg and 75.2 ± 6.84 kg, respectively. At the end, final BW was 111.0 ± 4.97 kg for treatment 1 and 110.0 ± 6.31 kg for treatment 2. The total trial duration in treatment 1 was significantly shorter that in treat 2 (33 days vs. 39 days; $P<0.01$). Average daily gain (ADG) was significantly greater in treatment 1 compared to treatment 2 (1090 g/day vs. 890/day, $P<0.01$). Linear regression showed a strong correlation between CT-estimated body mass including fat, muscle and bone with the initial live BW ($y = 0.8128x - 2.5451$; $R^2 = 0.922$) and end live BW ($y = 0.5925x + 21.935$; $R^2 = 0.606$). At the initial CT scanning, body fat content was 18.4% and 19.1%, body muscle content was 51.6% and 50.1%, and bone content of both treatments was 8.3%, and the rest fraction content was 21.8% and 22.5% for treatment 1 and 2, respectively. At the end, body fat content was 22.6% and 23.7% ($P<0.01$), body muscle content was 48.7% and 45.2% ($P<0.01$), bone content was 8.8% and 8.9%, and the rest content was 20.1% and 22.0% for treatment 1 and 2, respectively. The CT technology seems a suitable method to assess the body composition in pigs under different feeding strategies.

Funding
This research received no external funding.

Quantification of ATP in peptide bond formation from a chemical property-based approach

K.M. Kennedy[1], H.A. Rossow[2]
[1]Utah State University, Logan, USA. [2]University of California - Davis, Davis, USA
(katherine.kennedy@usu.edu)

Estimating the energetic cost of protein synthesis is important for determining the requirements of animals and energetic research. It is generally well-accepted that the cost to generate a peptide bond, a condensation reaction, is approximately 4 to 5 energy equivalents (ATP/GTP). However, we propose that a mathematical approach should be possible to determine the minimum number of moles of ATP required to form a peptide bond based on the heat of combustion for various peptides and amino acids and the energy associated with forming and expending one mole of ATP. Moles of ATP per peptide bond = $(((\Delta H^{\circ}_{c(protein)} + (\Delta H^{\circ}_{f(water)} \times PB)) - \sum\Delta H^{\circ}_{c(AA)}) - (\Delta H^{\circ}_{vap(water)} \times PB)) \div (PB \times \Delta H_{L(ATP)})$ where $\Delta H^{\circ}_{c(protein)}$ is the heat of combustion of the protein (kJ/mole), $\Delta H^{\circ}_{f(water)}$ is the heat of formation of water (-285.8 kJ/mole), PB is the number of peptide bonds (peptide bond) in the protein, $\Delta H^{\circ}_{c(AA)}$ is the heat of combustion of the intact amino acid (kJ/mole), $\Delta H^{\circ}_{vap(water)}$ is the heat of vaporization of water (40.65 kJ/mole), and $\Delta H_{L(ATP)}$ is the heat loss associated with the formation and use of one mole of ATP (kJ/mole ATP). The heat of combustions for various peptides and AA were those reported by Domalski (1972) and Yang et al. (1999). The $\Delta H^{\circ}_{vap(water)}$ is subtracted to account for the energy loss associated with the generated water molecules. We assume that 1 mole of H_2O is formed per peptide bond. With the discovery that ATP synthase in mammals contains 8 c-proteins, improvements to estimates of ATP yield by oxidative phosphorylation for various metabolites have improved. Under this assumption and others, we estimate that the number of ATP generated during the complete oxidation of 1 mole of glucose equals 32.9 ATP. Assuming that the energy for protein synthesis is generated from glucose, the energy associated with the formation and use of a mole of ATP is estimated as -85.7 kJ/mole (2820 kJ/mole glucose ÷ 32.9 ATP/ mole of glucose). Different energy sources and metabolic pathways likely impacts estimates. The number of ATP per peptide bond using the above assumptions were calculated for 12 peptides using our equation. The heat associated with $((\Delta H^{\circ}_{c(protein)} + (\Delta H^{\circ}_{f(water)} \times PB)) - \sum\Delta H^{\circ}_{c(AA)})$ is -311 ± 13.5 kJ/mole, which reversely resembles the bond energy of a C-N bond (~305 kJ/mole). The average number of ATP per peptide bond was 4.10 ± 0.16 moles. Peptides containing phenylalanine were consistently higher (range: 4.14 to 4.40 moles of ATP) and serylserine was lower (3.86 moles of ATP). Further work is required to determine how the size of the protein and alternative oxidation sources impact estimates.

References

Domalski, E.S., 1972. Journal of Physical and Chemical Reference Data 1, 221-277. https://doi.org/10.1063/1.3253099

Yang, X.W., Liu, J.R., Gao, S.L., Hou, Y.D., Shi, Q.Z., 1999. Thermochimica Acta 329, 109-115. https://doi.org/10.1016/S0040-6031(99)00002-7

Functional analysis of faecal samples from cows fed different diets

S. Mewes[1], K. Heinichen[2], M.R. Wilkens[1]
[1]Institute of Animal Nutrition, Nutrition Diseases and Dietetics, Leipzig University, Leipzig, Germany. [2]Oberholz Farm for Teaching and Research, Leipzig University, Leipzig, Germany (mirja.wilkens@uni-leipzig.de)

In dairy cows, great dry matter intake (DMI) of diets characterised by a high energy density can result in an increased flow of fermentable polysaccharides to the hindgut, thereby influencing the microbiome and its products (Gressley et al., 2011). The aim of this ongoing study is to investigate the adaption of the faecal microbiome in dry and lactating dairy cows by analysing the response to different substrates. Faecal samples were obtained from six lactating dairy cows with an average DMI of 29 ± 4.0 kg (36.4% aNDFom, 24.8% starch) and six dry cows with a DMI of 13 ± 2.1 kg (48.7% aNDFom, 6.3% starch). Faecal samples were incubated for 24 h at 38°C either without substrate or after addition of 10% glucose (based on DM), starch or cellulose. Samples were taken at baseline, 6 h, 12 h and 24 h. The impact of substrate was addressed by calculating the difference between the pH in the samples incubated with glucose, starch or cellulose and the pH in the time control without substrate. A mixed-effects model (time, diet and interaction between time and diet) followed by Sidak´s post-test including correction for multiple comparisons was applied to reveal significant differences ($P < 0.05$). The absolute pH at baseline amounted to 6.52 ± 0.14 in faeces of lactating and 7.27 ± 0.40 in dry cows. A significant decrease over time was observed in all samples including the time control without addition of substrate. The effect of glucose in relation to the control on pH of faecal samples of lactating cows was significantly smaller than in faecal samples of dry cows (12 h: -1.35 *vs.* -1.93; 24 h: -1.31 *vs.* -1.83). In contrast, the decrease of pH after the addition of starch was more pronounced in faecal samples from lactating cows than in samples from dry cows (12 h: -0.66 *vs.* -0.27; 24 h: -1.07 *vs.* -0.64). However, due to high variances in the samples from lactating cows, these differences could not be verified statistically ($P = 0.058$, $P = 0.050$). Only minor changes could be observed after incubation with cellulose, with a significantly smaller decrease in faecal samples from lactating cows in comparison to those obtained from dry cows after 12 h (-0.09 vs. -0.23). These preliminary results indicate that the microbiome in faeces of lactating cows comprises more amylolytic and less cellulolytic species. We assume that the microbiome in faeces of dry cows is not adapted to degrade starch but more efficient in utilising cellulose, while glucose induces a more pronounced drop in pH due to a lack of lactate metabolising species. However, data on VFA, starch and lactate as affected by the incubation are needed to support this hypothesis.

Reference

Gressley, T.F., Hall, M.B., Armentano, L.E., 2011. Journal of Animal Science 89, 1120-1130. https://doi.org/10.2527/jas.2010-3460

Funding

We would like to thank the H. Wilhelm Schaumann Foundation for financial support.

Relationship between dynamic body acceleration and heart rate for estimating energy expenditure in dairy cattle

A. Casal[1], A. Jasinsky[1], A.C. Espasandin[1], M. Carriquiry[2]
[1]Facultad de Agronomia – Universidad de la Republica - EEMAC, Ruta3 km 363, 60000, Paysandu, Uruguay. [2]2Facultad de Agronomia – Universidad de la Republica, Av. Garzon 780, 12900, Montevideo, Uruguay (acasal@fagro.edu.uy)

Animal energy expenditure, or heat production (HP; MJ/day), is typically measured in controlled laboratory settings using calorimetric or respiratory chambers. However, these methods are unsuitable for grazing animals due to the influence of physical activity and environmental factors. Alternatives such as the heart rate (HR; beats/min) – O2 pulse method (indirect calorimetry; Brosh, 2007) exist; but HR-loggers are relatively difficult to deploy in commercial production systems. Thus, body acceleration measurements have emerged as a possible field technique for estimating HP (Miwa et al., 2015). The objective of this study was to test the potential use of an acceleration index (overall dynamic body acceleration; ODBA), for estimating the HP of dairy cows in a pasture-based system. Spring-calved multiparous Holstein cows (n=36) blocked according to average calving date, lactation number (2.7 ± 1.05), body weight (BW; 624±66 kg), and body condition score (BCS; 2.8±0.24) in 3 feeding systems: cows housed in a compost barn and fed a total mixed ration (TMR) ad libitum (CB-TMR, n = 12) and cows grazing (7-day rotational system on a mixed pasture with a daily herbage allocation of 27 kg DM/ cow) and supplemented with TMR in a compost barn (CB-GRZ, n = 12); or in an open-sky pen (OP-GRZ, n = 12) were used. All cows were milked twice daily, and milk production was recorded daily. Cow BW was recorded monthly, and BCS was assessed every two weeks. Heat production was measured at 135 ±14 days of lactation using the HR-O_2 pulse technique. Heart rate (RCX3, Polar Electro Oy, Kempele, Finland) and body accelerations (g) in 3-axes (HOBO® Pendant®G; (Onset Computer Corporation, Bourne, MA, USA) were recorded simultaneously for 5 days. Both data loggers were mounted on the animal through an elastic belt placed around the thorax and behind the forelegs. The data acquisition success rate was 63% pairs of HR and OBDA data were used for the analysis. Effect of ODBA on HR and HP were analyzed with a mixed model and prediction equations were developed. Correlations between ODBA and HR or ODBA and HP were significant and high ($P=0.03$; $r>0.88$ and $P=0.03$; $r=0.91$). The effect of feeding system and milk yield on HR and HP were not significant ($P > 0.20$), thus, equations for HR and HP were established for correcting only by BW ($HR_{PRED}=5.17_{ODBA}+ 0.072_{BW} + 20.0$; $R^2adj=0.74$; $HP_{PRED}=84.001_{ODBA}+ 1.38_{BW} -2.52$; $R^2adj=0.85$). In agreement, Miwa et al. (2015) using cattle, goats and sheep (n=20) reported a correlation between ODBA and HR of 0.86. Based on our and previous results, ODBA could be used as a good proxy for estimating the energy expenditure of grazing farm animals but more research is needed to use this accelerometry technique in field research and commercial farms settings.

References

Brosh, A., 2007. Journal of Animal Science 85, 1213-1227. https://doi.org/10.2527/jas.2006-298
Miwa, M., Oishi, K., Nakagawa, Y., Maeno, H., Anzai, H., Kumagai, H., Hirooka, H., 2015. PLOS ONE 10, e0128042. https://doi.org/10.1371/journal.pone.0128042

Funding

CSIC

Protipig: Unveiling the link between gastrointestinal microbiota and fecal enterotypes in pigs

N. Sarpong[1,2], J. Seifert[1,2], A. Camarinha-Silva[1,2]
[1]University of Hohenheim, Stuttgart-Hohenheim, Germany. [2]HoLMiR - Hohenheim Center for Livestock Microbiome Research, Stuttgart-Hohenheim, Germany (Naomi.Sarpong@uni-hohenheim.de)

The intestinal microbiome of pigs plays a significant role in feed conversion and digestion. However, studies using shotgun metagenomics to investigate the entire porcine gastrointestinal tract (GIT) remain limited. Enterotypes simplify fecal microbiota structure, but their ability to represent microbial communities throughout different GIT sections is unclear. This study aims to evaluate whether fecal enterotypes can infer bacterial composition in digesta across the GIT by integrating amplicon sequencing, shotgun metagenomics, and volatile fatty acid (VFA) analysis. Forty-eight male pigs were euthanized at 21 weeks of age (97.1 kg mean body weight) after being reared on a two-phase diet with marginal lysine supply (90% of recommended levels) (Berghaus et al. 2023). Digesta and mucosa samples were collected from stomach, duodenum, jejunum, ileum and cecum. The gastric mucosa was sampled at the *Pars pylorica* (PP), *Pars nonglandularis* and Cardiac glands zone (CGZ). Target amplicon sequencing of the 16S rRNA gene was performed on both sample types, while shotgun metagenomic sequencing and VFA analysis were conducted on digesta samples. Previously, fecal microbiota was characterized for all pigs of the entire project (n= 508) during both feeding phases (FP). Fecal samples (n = 892) were assigned to two enterotypes: Lactobacillus (cluster LACTO) and Clostridium sensu stricto (cluster CSST), as described in Sarpong et al. (2024). Enterotype stability across FPs was assessed, and shotgun metagenomic data from 45 pigs' digesta were assigned to enterotype subgroups. Target amplicon sequencing data showed significant differences in microbial composition between sample types within and across GIT sections (both sample types: $P < 0.001$). Pairwise PERMANOVA analyses of digesta and mucosa confirmed microbial differences across all GIT sections, except between CGZ and PP. Several genera, e.g. *Bifidobacterium* and *Prevotella* differed between sample types across all GIT sections ($P < 0.05$), while *Shuttleworthia* showed differences in the stomach and small intestine ($P < 0.05$). Shotgun metagenomics revealed that *Lactobacillus*, *Ligilactobacillus* and *Limosilactobacillus* species had their highest relative abundance in the stomach (60.8%), whereas *Streptococcus alactolyticus* was least abundant in the stomach (5.4%) but most abundant in the small intestine (duodenum 35.4%, jejunum 45.1% and ileum 44.8%). Acetate and butyrate concentrations were lowest in the duodenum and increased along the GIT ($P < 0.05$), while iso-butyrate and iso-valerate concentrations began rising in the ileum ($P < 0.05$). At the species level, PERMANOVA identified enterotype-associated bacterial differences in the stomach, ileum, and cecum ($P < 0.05$). *Lactobacillus amylovorus* differed between enterotype subgroups across these sections ($P < 0.05$), and *Streptococcus alactolyticus* showed enterotype-related variation only in the ileum ($P < 0.05$). Our findings reveal a unique microbial profile in the gastric mucosa that differs markedly from other samples. It also shows that fecal enterotype classification applies to digesta, offering a practical method for microbiome-based livestock research.

References

Berghaus, D., Haese, E., Weishaar, R., Sarpong, N., Kurz, A., Seifer, J., Camarinha-Silva, A., Bennewitz, J., Chillon, T., Stefanski, V., 2023. Animal 17, 100424. https://doi.org/10.1016/j.animal.2023.100424
Sarpong, N., Seifert, J., Bennewitz, J., Rodehutscord, M., Camarinha-Silva, A., 2024. Frontiers in Microbiology 15, 1354537. https://doi.org/10.3389/fmicb.2024.1354537

Funding

The study was funded by the H. Wilhelm Schaumann Stiftung.

Wool and cerumen dioxin and volatile organic compound analyses as non-invasive proxies of adipose tissue dioxin level in ewes

S. Lerch[1], J. Ratel[2], R. Siegenthaler[3], F. Dohme-Meier[1], E. Engel[2], M. Zennegg[4]
[1]Ruminant Nutrition and Emissions, Agroscope, 1725 Posieux, Switzerland. [2]INRAE, UR QuaPA, 63122 Saint-Genès Champanelle, France. [3]Research Contracts Animals, Agroscope, 1725 Posieux, Switzerland. [4]Empa, Analytical Center, 8600 Dübendorf, Switzerland (sylvain.lerch@agroscope.admin.ch)

In case of suspicion of herd contamination, monitoring the polychlorinated dibenzo-*p*-dioxin and dibenzofuran (PCDD/F) level in livestock prior to slaughter is mandatory to alleviate disposal of carcasses. The reference method for quantifying *in vivo* the contamination level is to make a biopsy of adipose tissue followed by target PCDD/F analysis, which is quite invasive, time-consuming and costly. To overcome such limitations, the development of non-invasive proxies of the body contamination level is required. In sheep, sampling of wool or cerumen are promising options, and non-target analyses offer innovative proxies of contamination status. In such case, alternative omics and chemometric techniques are combined to detect specific metabolic fingerprints of pollutant exposure. The aim was to assess the reliability of target and non-target (i.e. profile in volatile organic compounds, VOCs) analyses on wool and cerumen as proxies of adipose tissue PCDD/F level. Details of the experiment are presented in Lerch *et al.* (2024). In brief, six suckler ewes ("Roux du Valais", 4.7±1.6 years old, 53.9±9.6 kg body weight) were formerly exposed (from birth) to PCDD/F through feeding of pasture and hay from soil-polluted grasslands of the Lausanne area (Switzerland). They were further depurated after being switched to a non-contaminated hay from day 29 of lactation. Four additional ewes served as control and were fed continuously with non-contaminated hay. At depuration days 0 (end of the exposure period), 32, 60 (weaning), 130 and 188 (slaughter), milk, sternal adipose tissue, wool and cerumen were sampled for PCDD/F analysis by Atmospheric Pressure Gas Chromatography-Mass Spectrometry. At depuration days 0, 60 and 188, milk, wool and cerumen were analyzed for VOC profile by Solid Phase Micro Extraction-Gas Chromatography-Mass spectrometry (Ratel *et al.*, 2022). Linear regressions were set between PCDD/F concentrations in the different tissues, whereas VOC data were analyzed by principal component analysis. Target analysis of PCDD/F in wool allowed a fair estimation of the corresponding contamination level in adipose tissue (for the sum toxic-equivalent in reference to 2,3,7,8-TCDD: R^2= 0.85, mean bias = +1.2 pg g^{-1} lipids) at moderate and high PCDD/F concentrations (2-30 pg toxic-equivalent g^{-1} lipids). At lower PCDD/F level, external contamination of wool, presumably through air deposition, may explain poorer concordance between wool and adipose tissue concentrations. Conversely, very low amount of cerumen collected from individual ewes (34-130 mg extracted-lipids), was not sufficient to perform reliable PCDD/F analysis, but allowed VOC analysis. Milk, cerumen and especially wool allowed distinguish between exposed, depurated and non-exposed ewes from their VOC signatures. Due to the ease of non-invasive sampling, the potential of wool, and to a lesser extent of cerumen, as monitoring tool for estimating the contamination status of sheep was confirmed.

References

Lerch, S., Corde, S., Rawel, H.M., 2024. Journal of Agricultural and Food Chemistry 72, 14941-14955. https://doi.org/10.1021/acs.jafc.4c02626
Ratel, J., Chevalier, C., Debrauwer, L., Nguyen-The, C., Karam, M., 2022. Food Chemistry 374, 131504. https://doi.org/10.1016/j.foodchem.2021.131504

Funding The project ProxyPOP (n°4.23.03) was co-funded by the "Direction Générale de l'Environnement (DGE)" of canton Vaud (Lausanne, Switzerland) and the Federal Food Safety and Veterinary Office (FSVO, Bern, Switzerland). Lausanne municipality supported the project by providing animals, forages and human resources required for the conduction of the ewe experiment.

Predicting body fat levels of laying hens using near-infrared spectroscopy

Lieske van Eck[1,2], I. Carvalhido[1], E. Margaria[3], H. Chen[1]
[1]Cargill Animal Nutrition, Velddriel, Netherlands. [2]Wageningen University, Wageningen, Netherlands. [3]Cargill Animal Nutrition, Elk River, USA (lieske_van_eck@cargill.com)

Laying hen body composition is influenced by diet, it is an indicator of the energy balance of hens, and it might influence long-term egg production. Evaluating body fat levels to adapt diet formulation could therefore improve sustainable egg production (van Eck et al., 2023). The objective of this study was to develop a tool to measure laying hen body fat levels non-invasively, by creating a model that correlates Near-InfraRed (NIR) spectroscopy with abdominal fat pad weight in laying hens. A hand-held NIR scanner with a wavelength ranging between 740 and 1070 nm was used (SCiO™;Consumer Physics). NIR measurements were performed on 734 female laying hens of four flocks with five breeds (Hy-Line White and Brown, Lohmann White and Brown, Dekalb White), ranging between 15 and 90 weeks of age over a time period of 4 years. To create variation in fat pad weights, hens received diets with energy ranging between 2625 kcal/kg and 2800 kcal/kg and apparent fecal digestible lysine levels ranging between 0.58% and 0.72%. Each hen was scanned three times by manually holder her upside down, without any anaesthesia, and putting the scanner on the skin, between the keel bone and cloaca. Feathers were pulled aside but not removed. Within a day and after scanning, hens were euthanized and dissected to measure the abdominal fat pad weight (ranging from 0.3 g to 99.3 g). A machine learning random forest algorithm was used to correlate the spectra with *ex-vivo* fat pad weights. In general, body fat pad weight was higher in brown than in white hens and increased with age from week 15 until week 35 (brown hens +66.7g; white hens +45.8g), remained relatively stable until week 55 (brown hens -4.7g; white hens -8.5g), after which it decreased more rapidly (brown hens-32.1 g; white hens -14.2g). The coefficient of determination (R^2) of the created model was 0.699, the slope 0.695 and the RMSE was 14.8g. A set of 50 independent additional hens were scanned and dissected to validate the model. Statistics of the validation results showed an R^2 of 0.690, a slope of 0.800 and an RMSE of 15.44g. These results show a moderate correlation, lower in comparison to the currently most accepted alternative of Dual-energy X-ray (DEXA) used for broilers and breeders (Schallier et al., 2019). The benefit of using NIR over DEXA, however, is that it is faster, cheaper, and hens do not have to be sedated, which means it is truly non-invasive. We therefore conclude that NIR can be used as a non-invasive technique to measure body fat level of laying hens in the field. This allows nutritionists to make more informed decisions on diet formulation to improve laying performance and efficiency.

References

van Eck, L.M., Enting, H., Carvalhido, I.J., Chen, H., Kwakkel, R.P., 2023. World's Poultry Science Journal 79, 243-264. https://doi.org/10.1080/00439339.2023.2189206
Schallier, S., Li, C., Lesuisse, J., Janssens, G.P.J., Everaert, N., Buyse, J., 2019. Poultry Science 98, 2652-2661. https://doi.org/10.3382/ps/pez013

Utilizing gas flux to estimate dietary energy values in *Bos taurus* cattle under different management systems

J.T. Silva, A.K. Schilling-Hazlett, L.M. Campos, K.R. Stackhouse-Lawson, S.E. Place, J.J. Vargas, P.H.V. Carvalho
Colorado State University, Fort Collins, USA (julia.travassosdasilva@colostate.edu)

The objective of this study was to evaluate the precision, accuracy, and agreement among growth performance-adjusted and gas-adjusted methods for estimating net energy for maintenance (NEm) and gain (NEg) in *Bos taurus* cattle under technology and non-technology management systems. Estimation of NEm and NEg was derived using individual live growth performance (LpaNEm and LpaNEg), carcass data (CpaNEm and CpaNEg), and gas flux measurements (gaNEm and gaNEg) using an automated head chamber system (AHCS; GreenFeed) to collect methane, carbon dioxide, and oxygen. Methods varied by using either live BW or adjusted-final BW, which was calculated from carcass characteristics to account for differences in tissue composition and energy content. Heat production was estimated using Kaufmann et al. (2011) or Brouwer (1965) equations. Eighty-yearling Angus steers were randomly assigned to one of two management systems, technology [TRT; n = 43; (BW) = 498 ± 27 kg] and non-technology [CON; n = 37; BW = 478 ± 24 kg]. Steers in TRT received growth-promoting hormonal implants and were supplemented with ionophore, antibiotic, and beta-agonist (last 42 days), while steers in CON received no growth-promoting technologies, ionophores, or antibiotics. Steers were housed in two pens, 1 pen/treatment, and each pen was equipped with one AHCS. Steers were acclimated to AHCS for 7 d before the initiation of data collection. Steers were offered a finishing diet with 2.04 NEm and 1.43 NEg Mcal/kg DM. Animals were individually weighed at the beginning of the experiment to determine the initial BW and at the end (96 d) of the experimental period to determine the final BW, and all animals were shipped to a commercial packing facility for slaughter and carcass characteristics data collection. Data were analyzed using the statistical software R (v.4.4.2), which considered the animal as the experimental unit. Lin's concordance correlation coefficient (CCC), Pearson correlation (r), and root mean square error of prediction (RMSEP) were calculated to assess agreement, accuracy, and precision of the predictions. The LpaNEm, LpaNEg, CpaNEm, and CpaNEg exhibited moderate to high agreement with gaNEm and gaNEg across both management systems. Specifically, the CCC ranged from 0.63 (LpaNEg under the Brouwer method) to 0.74 (CpaNEg under the Kaufmann method). At the same time, r varied between 0.72 (LpaNEg under the Brouwer method) and 0.81 (CpaNEm under the Kaufmann method). The RMSE% ranged from 5.21% (LpaNEm under the Kaufmann method) to 10.26% (LpaNEg under the Brouwer method), indicating a reasonable level of prediction accuracy. These results suggest that researchers can implement the AHCS to provide reliable estimates of dietary energy values in finishing *Bos taurus* cattle across different management systems. By measuring gas exchange in real-time, the AHCS provides a valuable tool for optimizing nutrition strategies and maximizing feed efficiency in a feedlot.

References

Brouwer, E. 1965. Report of sub-committee on constants and factors. In: Proceedings of the 3rd Symposium on Energy Metabolism. Troon (Scotland): European Association of Animal Production Publication; p. 441–443.

Kaufmann, L.D., Münger, A., Rérat, M., Junghans, P., Görs, S., Metges, C.C., Dohme-Meier, F., 2011. Journal of Dairy Science 94, 1989-2000. https://doi.org/10.3168/jds.2010-3805

Repurposing food losses for pigs: a sustainable feed solution without compromising animal behaviour

M. Tretola[1], A. Stratmann[2], S. Mazzoleni[3], F. Mercogliano[4], C. Di Lorenzo[5], S. Barbieri[3], G. Bee[1], L. Pinotti[3]
[1]Swine Research Group, Agroscope, Posieux, Switzerland. [2]Animal Welfare Group, Agroscope, Posieux, Switzerland. [3]Department of Veterinary Medicine and Animal Science, University of Milan, Lodi, Italy. [4]Department of Pharmacological and Biomolecular Sciences, Università degli Studi di Milano, Milan, Italy. [5]Department of Pharmacological and Biomolecular Sciences, University of Milan, Milan, Italy (marco.tretola@agroscope.admin.ch)

Former foodstuff products (FFPs) are promising alternative feed ingredients that help prevent food waste and reduce the environmental impact of food production. However, concerns exist regarding their potential effects on animal behavior and welfare, particularly due to the presence of caffeine, theobromine, and ultra-processed sugars in confectionery-based FFPs. This study evaluates the behavioral responses of thirty-six Swiss Large White male castrated pigs fed diets incorporating salty and sugary FFPs during the growing (G) and finishing (F) phases. Pigs were assigned to one of three dietary treatments: (1) a standard diet (ST-G, ST-F) with 0% FFPs, (2) a diet where 30% of conventional ingredients were replaced by sugary FFPs (SU-G, SU-F), and (3) a diet where 30% of conventional ingredients were replaced by salty FFPs (SA-G, SA-F). Behavioral traits—including social interactions, exploratory behavior and aggression—were assessed using continuous video recordings at eight time points throughout the trial. Additionally, skin and tail lesions were monitored to evaluate potential welfare implications. Key behavioral ratios were analyzed using linear mixed-effects models with treatment, time, and their interaction as fixed effects, and Pig ID as a random factor. Collinear and rare variables were excluded. Log transformation was applied where needed. Lesion scores were analyzed using one-way ANOVA, with repeated measures for time-based data. Significance was set at $p < 0.05$. Analyses were performed in R (v4.2.1). Pigs receiving the sugary and salty feed diets reduced ($P=0.045$) lying in contact over time in comparison to pigs that received the standard feed. Pigs receiving the SA feed had a higher ($P=0.002$) eating ratio (i.e., were observed eating more often) compared to the other two groups. No significant differences ($P > 0.05$) between the experimental groups and the standard diet group in terms of abnormal behaviors or skin lesions were found. These findings indicate that replacing up to 30% of conventional feed ingredients with either salty or sugary FFPs does not negatively affect pig behavior, health, or welfare. This supports the feasibility of integrating FFPs into pig diets as a sustainable feeding strategy, contributing to circular agriculture while maintaining animal well-being. Further research should explore long-term effects on growth performance, metabolic adaptations, and meat quality to optimize the use of FFPs in livestock nutrition.

Impact of sanitary conditions and self-supplementation of dietary amino acids on performance, behaviour, and brain monoamine levels of piglets in a choice-feeding setting

I. Minussi[1], W.J.J. Gerrits[1], A.J.M. Jansman[2], G. Troupakis[3], M. Diepeveen - de Bruin[4], Y. Vermeiren[4], J.E. Bolhuis[3]

[1]Animal Nutrition Group, Wageningen University & Research, Wageningen, Netherlands. [2]Wageningen Livestock Research, Wageningen University & Researcg, Wageningen, Netherlands. [3]Adaptation Physiology Group, Wageningen University & Research, Wageningen, Netherlands. [4]Nutritional Biology Group, Wageningen University & Research, Wageningen, Netherlands (walter.gerrits@wur.nl)

Immune activation due to poor hygienic conditions may affect behaviour, as the immune system and brain are linked through several mechanisms. This putative effect may partly result from increased amino acid (AA) metabolism, affecting brain monoamine levels. As pigs can select a diet that meets their AA requirements, offering them the choice between an AA deficient and AA enriched diet may influence the effects of sanitary conditions on brain neurochemistry and behaviour. We investigated the behavioural and neurochemical effects of high (HSC) vs. low (LSC) sanitary conditions on pigs with or without a choice to select between two diets differing in AA concentration in a 2x2 factorial experiment. Female pair-housed piglets (n=48) were kept under HSC or LSC and were offered either a diet deficient in eight indispensable AA or the choice between the deficient diet and a diet enriched with these AA for 19 days. Behaviour, salivary cortisol, blood tryptophan (Trp) and serotonin (5-HT), and hippocampal/prefrontal concentrations of Trp, related monoamines and their metabolites were analysed. All parameters were analyzed with linear mixed models with fixed effects of sanitary conditions, dietary choice, their interaction, batch (with day included for salivary cortisol), and a random effect of pen. Behaviour was analysed with a generalized linear mixed effects model with a Poisson distribution and Log link function for frequencies or a binomial distribution and logit link function, and a multiplicative overdispersion parameter, with sanitary conditions, dietary choice, their interaction, and batch as fixed effects. LSC had lower platelet serotonin and plasma Trp than HSC piglets. Hippocampal and prefrontal cortex concentrations of Trp, kynurenine (KYN), kynurenic acid, epinephrine, norepinephrine, 5-HT, dopamine (DA), 5-hydroxyindoleacetic acid and homovanillic acid (HVA) were lower for LSC than HSC piglets. LSC piglets had lower KYN/Trp ratios in the hippocampus and, if without dietary choice, in the prefrontal cortex. LSC piglets showed less play behaviours. Piglets without a dietary choice had lower plasma Trp, higher hippocampal HVA/DA turnover, and spent more time on damaging behaviours. No effects on salivary cortisol were found. A decline in health status induced by poor hygienic conditions reduced platelet 5-HT, blood and brain Trp, as well as brain neurotransmitter concentrations, and negatively influenced behaviour of pigs. Milder behavioural effects were observed when offering a choice between an AA deficient and enriched diet.

Funding

This study is part of the Public Private Partnership LWV19175 funded by the Dutch Ministry of Agriculture, Nature and Food, De Heus, ForFarmers, and Eurolysine. Gerrit Grijns Initiative (GGI) seeding grant is also acknowledged. Funders had no role in data collection, analysis, decision to publish, or preparation of the manuscript.

Effects of increased number of daily meals on ileal digestibility of calcium and phosphorus in sows

K.K. Lyderik[1], C. Marcussen[2], T. Thymann[1], S.M. Offersen[1], A.V. Strathe[1]
[1]Department of Veterinary and Animal Science, University of Copenhagen, Frederiksberg, Denmark. [2]Department of Veterinary Clinical Sciences, University of Copenhagen, Frederiksberg, Denmark (avst@sund.ku.dk)

Utilization of calcium (Ca) and phosphorus (P) are low in gestating sows relative to growing pig and lactating sows. For practical reasons many gestating sows in Denmark are only fed one meal per day. However, it is not known if the digestibility of Ca and P are dependent on the number of daily meals. The aim of the study was, therefore, to test the effect of feeding sows 1, 2 or 3 meals per day on apparent ileal digestibility (AID) of Ca and P. Five empty sows (Yorkshire x Landrace) were fitted with T-cannulas in the distal ileum (Stein et al., 1998). Sows were allowed 7 days for postsurgical adaptation before the experimental feeding was initiated. All sows were fed the same daily amount of feed (2.15 kg/day, 25.4 MJ ME/day, 15.2 g Ca/day, 8.0 g P/day), given as either one daily ration (2.15 kg), or divided into two (1.075 kg) or three meals (0.717 kg) per day. The diet was based on barley, wheat, sugar beet pellets and soy bean meal, and yttrium oxide (0.1%) was added as indigestible marker. The effect of daily meals was examined in a cross-over design, where all sows were allotted to all dietary treatments and there was 8 replicates per group. One treatment period lasted seven days, where the first five days were adaptation to the new treatment and on days 6 and 7, samples of ileal digesta were collected. Feed and ileal digesta were analyzed for nutrients and yttrium. The AID was calculated using the equation described by Fan et al. (1995). Data for AID of Ca and P were analyzed with ANOVA in R in a randomized block design. Sow was regarded as the experimental unit. The statistical model included the number of daily meals as a fixed effect and sow and period as random effects. The number of daily meals did not affect AID of Ca ($P=0.16$) and P ($P=0.18$) in the sows. AID of Ca was -16.1, -15.1 and 3.1%, respectively in sows fed 1, 2 or 3 meals per day. AID of P was 9.9, 7.2 and 24.2 % respectively in sows fed 1, 2 or 3 meals per day. The negative values indicate that the endogenous loss of Ca is larger than the absorption of Ca. The very low AID found in the current study in empty sows could be a result of a low Ca and P requirement by these sows. In conclusion, increased number of daily meals did not affect the utilization of Ca and P in sows, indicating that at the present volume of feeding, the gut absorptive mechanisms for Ca and P are regulated independent of the frequency of feeding.

References

Fan, M.Z., Sauer, W.C., McBurney, M.I., 1995. Journal of Animal Science 73, 2319-2328.
https://doi.org/10.2527/1995.7382319x
Stein, H.H., Shipley, C.F., Easter, R.A., 1998. Journal of Animal Science 76, 1433-1436.
https://doi.org/10.2527/1998.7651433x

Funding

The Danish Pig Levy Foundation

Genomic architecture of vitamin D metabolism and bone turnover in pigs

D. Paul[1], H. Reyer[1], M. Oster[1], S. Ponsuksili[1], K. Wimmers[1,2]
[1]Research Institute for Farm Animal Biology (FBN), Dummerstorf, Germany. [2]Faculty of Agricultural and Environmental Sciences, University Rostock, Rostock, Germany (reyer@fbn-dummerstorf.de)

Pigs achieve calcium (Ca) and phosphorus (P) homeostasis through complex physiological mechanisms. Physiological Ca levels are essential for blood clotting, muscle contraction, and enzymatic activities, while P is crucial for nucleic acid synthesis, ATP production, and blood pH buffering. Moreover, bone health is influenced by mineral metabolism, which is maintained by endocrine regulators including vitamin D metabolites (calcidiol as storage form; calcitriol as biologically active form) that modulate mineral absorption, deposition, and excretion. Genetic factors play a significant role in mineral efficiency and bone integrity, with studies suggesting that nearly half of the variation in blood P levels in pigs is genetically determined. The current study aims to investigate the genetic determinants of the inter-individual variability of vitamin D metabolites and bone remodeling markers in pigs. Therefore, serum calcidiol (25-hydroxyvitamin D3), calcitriol (1,25-dihydroxyvitamin D3), β-CTX (C-terminal telopeptide), and CICP (type I C-terminal collagen propeptide) levels were analyzed in a population of 610 purebred German Landrace pigs aged 166 ± 20 days and genotyped for 60k SNPs. Pigs exhibited significant natural variation in physiological parameters related to maintaining mineral homeostasis and growth. Genome-wide association studies (GWAS) were conducted using the Enriched Compressed Mixed Linear Model in GAPIT v3 to map significantly associated SNPs and putative candidate genes. Significantly associated SNPs with serum calcidiol were identified on pig chromosomes 2, 8, 9, 12, and 13 harboring in total 13 annotated genes. These comprise putative functional candidate genes explaining the inter-individual variability in vitamin D such as *GC*, *ALB*, *AFP*, *PTH*, and *ABCC3*. In blood, vitamin D metabolites are predominantly bound to the vitamin D-binding protein (VDBP), which is encoded by the GC gene and accounts for 85-90% of the calcidiol transport. The remaining 10-15% of vitamin D transport is facilitated by albumin (encoded by *ALB*) and other lipoproteins. Parathyroid hormone (PTH) plays a fundamental role in vitamin D activation by stimulating 1α-hydroxylase, the enzyme responsible for converting calcidiol into calcitriol in the kidneys. The protein encoded by the *ABCC3* gene, a potential regulator of bile acid transport that may interact with vitamin D metabolites, is known to be under the regulation of the Vitamin D response element (VDRE). The significantly associated gene regions, especially considering the biological role of the identified candidate genes, implicate in particular transport mechanisms of vitamin D metabolites as major determinants of the variability of the vitamin D system. This study represents the first comprehensive analysis of vitamin D metabolites, including serum calcidiol and serum calcitriol, alongside bone remodelling markers such as serum β-CTX and serum CICP in pigs. Further investigation is required for diagnosing and predicting individual characteristics related to mineral utilization and bone development, ultimately contributing to the enhancement of animal health and sustainable livestock farming.

Funding

This work was financially supported by the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) – Project number 503753038.

Effect of phytase supplementation on growth performance, bone ash, and ileal and faecal mineral digestibility on growing pigs fed diets without inorganic phosphorus supplementation

R. Lizardo[1], J. Vázquez[1], B.R. Villca[1], G. Gomes[2], G. Cordero[2]
[1]IRTA - Animal Nutrition, Constantí, Spain. [2]ABVista, Madrid, Spain (rosil.lizardo@irta.es)

Phytic acid (inositol hexa-kis phosphate, InsP6) is present in most plant-based feed ingredients, and it may be considered as antinutritional due to the negative charges carried by the 6 phosphate groups arranged around the inositol ring. In the absence of added exogenous phytase, the extent of InsP6 hydrolysis at the terminal ileum of swine is quite limited (Schlemmer et al., 2001). Phytase application has evolved significantly in the last years, with higher dose rates leading to a greater reduction in inorganic P supply. An experiment was conducted to determine the effect of phytase on growth performance, ileal (AID) and faecal (ATTD) mineral digestibility and bone mineralization in growing pigs. One hundred fifty male and female Pietrain*(Landrace*Large White) pigs of 8.42 kg liveweight were used. They were distributed into 10 blocks according to sex and body weight and allocated at 3/pen in 50 pens for a 35-d trial. Intra-block they were randomly distributed into 5 dietary treatments corresponding to positive (PC: 0.35 % digestible P) and negative (NC: 0.12 % digestible P) control diets or the NC supplemented with 300, 600 or 1000 FTU/kg of phytase (Quantum Blue, AB Vista), respectively. Diets were wheat-barley-rice by-products and soyabean meal based and contained a tracer to determine digestibility. All the diets were pelleted and offered *ad libitum*. Faeces samples were collected at the end to analyse dry matter (DM), ash, Ca and P. Fifty piglets (1/pen) have been euthanised and bone metacarpals (III, IV) were sampled to determine bone ash, Ca and P contents. Ileum digesta from the last 120 cm of the ileum valve was collected to determine nutrient AID. Data were analysed by GLM procedure of SAS, and the means separated using Tukey's test ($P \leq 0.05$). Removal of inorganic-P supplementation negatively affected growth performance and feed efficacy ($P<0.01$) while the inclusion of phytase, regardless of the dose, restored it ($P<0.01$). Inclusion of phytase into diets improved ATTD of DM, ash, and Ca relative to treatments without phytase ($P<0.01$), whereas ash and P ATTD was proportionally increased by the dose of phytase used ($P<0.01$). Ash AID improved ($P<0.01$) relatively to the NC diet or with phytase inclusion while AID of P was positively affected ($P<0.01$) relative to both NC and PC diets. However, only a tendency to improve AID of Ca was observed ($P=0.08$). Metacarpal weight and ash content was negatively affected by P levels ($P<0.01$) while the inclusion of phytase restored it at the highest dose ($P<0.01$). In conclusion, the inclusion of phytase allows reducing the incorporation of P-inorganic in diets without affecting productive parameters, mineral digestibility, and bone mineralization, thus reducing the environmental impact of pig slurry and contribute to sustainability of swine production.

Reference

Schlemmer, U., Jany, K.D., Berk, A., Schulz, E., Rechkemmer, G., 2001. Archives of Animal Nutrition 55, 255-280. https://doi.org/10.1080/17450390109386197

Effects of a consensus bacterial 6-phytase variant on growth performance and bone mineral composition of wean-to-finish pigs fed diets with reduced energy and nutrient contents

P. Aymerich[1], R. Lizardo[2], B. Villca[2], D.E. Velayudhan[1], E. Vinyeta[1], Y. Dersjant-Li[1]
[1]Danisco Animal Nutrition & Health (IFF), Oegstgeest, Netherlands. [2]IRTA - Animal Nutrition, Constantí, Spain (pau.aymerich@iff.com)

A novel consensus bacterial 6-phytase variant (PhyG) has shown to improve amino acid (AA) and energy digestibility in swine as result of a quick break-down of phytate in the stomach (Espinosa et al, 2022). The aim of this experiment was to determine the effect of applying a full phytase matrix [standardized total tract digestible (STTD) P, Ca, net energy (NE) and standardized ileal digestible (SID) AA] on growth performance and bone composition of wean-to-finish pigs. A total of 180 weaned pigs [(LW x LD) x Pietrain; initial body weight (BW) 8.2±0.87 kg, 50:50 entire males and females] were distributed into 3 treatments with 20 replications (3 pigs/pen, single sex pens). Pelleted diets were based on corn, soybean byproducts, rice byproducts and wheat middlings and fed in 5 phases: 7-11, 11-25, 25-50, 50-75 and 75-110 kg BW. Treatments included: PC) nutritionally adequate positive control; NC1+1500FTU) reduced nutrient diet from PC (downspec: 0.18% STTD P, 0.20% Ca, 57-66 kcal NE/kg, 0.018-0.029% SID Lys) including PhyG at 1500 FTU/kg; NC2+2500FTU) reduced nutrient diet (downspec: 0.19-0.20% STTD P, 0.21% Ca, 59-68 kcal NE/kg, 0.019-0.031% SID Lys) including PhyG at 2500 FTU/kg. Both reduced nutrient diets had lower soybean meal and higher fibrous byproducts inclusion compared to PC. Pigs were marketed at days 125 and 139 of the trial, 60 and 120 pigs each, respectively. At the slaughterhouse, the left forefeet were collected to extract the metacarpal III and measure bone mineral contents. Data was analyzed in a mixed model including treatment, sex and their interaction as fixed effects, and block as random effect. Bone data was analyzed including final BW as a linear covariate. Orthogonal contrasts were used to determine the effect of phytase and phytase dosage. Phytase treatments (applied full matrix) numerically improved BW throughout the experiment (P>0.10), with final BW numerically increasing from 111.5 kg in PC to 113.5 kg in NC2+2500FTU. This was the result of a numerical improvement in feed intake from 1.60 in PC to 1.64 kg/d in both phytase groups (P=0.121). No significant differences in feed efficiency were observed (P>0.10). Regarding bone mineral composition, on average phytase treatments showed a 4.4% increase in metacarpal ash content (P<0.01) and 3.9% in the percentage of P in ash (P < 0.05) compared to PC. In addition, the metacarpal dry weight relative to BW was increased from 162 to 172 mg/kg with increasing phytase dosage (P<0.05). In conclusion, a full matrix application including STTD P, Ca, NE and SID AA can be used in wean-to-finish pigs without compromising growth performance and significantly improving bone mineral composition, enhancing the economic and environmental sustainability of pig production by using less costly and more circular feed ingredients.

Reference

Espinosa, C.D., Torres, L.J., Velayudhan, D.E., Dersjant-Li, Y., Stein, H.H., 2022. Journal of Animal Science 100, 12. https://doi.org/10.1093/jas/skac364

Effect of forage conservation on carotenoid and tocopherol content and composition

B.T. Diallo[1,2], B. Graulet[1], A. Boudon[3], D. Andueza[1], G. Maxin[1]
[1]INRAE, Saint-Genès-Champanelle, France. [2]Animine, Annecy, France. [3]INRAE, Saint-Gilles, France (boubacar-telly.diallo@inrae.fr)

Micronutrients like carotenoids and tocopherols play an essential role in ruminant production, health, reproduction. They are supplied by forages in which concentrations are known to vary with plant species, botanical composition, and vegetative stage. Moreover, additional variations could occur in conserved forages during processes and storage, but data on this are scarce. The aim of this trial was to evaluate the effect of forage conservation on carotenoid and tocopherol content and composition. The trial was conducted at INRAE Herbipôle research farm (Saint-Genès-Champanelle, France). Three neighbouring plots (permanent pasture, sainfoin and orchardgrass) were used, corresponding to species. Each plot was split into three areas as replicates. All areas were harvested at the end of May 2023. Then, grass was separated into green forage and four conserved forages (unwilted silage, wilted silage, haylage and hay) representing forage types. Unwilted silage was made just after cutting whereas wilted silage, haylage and hay were made after wilting grass on ground. Samples from conserved forages were collected after 4 months. All samples were freeze-dried and ground to less than 1mm before analysis. Carotenoids (neoxanthin, violaxanthin, antheraxanthin, zeaxanthin, β-cryptoxanthin, lutein and lutein epoxide, (9Z)-β-carotene, (13Z)-β-carotene and all-trans-β-carotene) and tocopherols (α- and γ-vitamers) were quantified after organic solvents extraction then liquid chromatography separation according to Chauveau-Duriot et al., (2010). Data were analyzed by ANOVA model including forage types, species and their interaction as fixed effects, and area as random effect. Total carotenoids and total tocopherols varied with forage type. Their contents in green forage (on average 420.2 and 53.7 µg/g DM respectively) decreased significantly ($P<0.001$) in conserved forages. Lowest values were observed in hay with average reductions of 83% for carotenoids and 59% for tocopherols per comparison to green forage, whereas silages and haylage had intermediate values. Among carotenoids, lutein represented more than half (54%) of total carotenoids. All molecules decreased in conserved forages except zeaxanthin and β-cryptoxanthin. Violaxanthin decreased the most, by 95% in conserved forages in average. Conversely, zeaxanthin increased by 65% in silages compared with green forage whereas β-cryptoxanthin did not vary between forages. Lutein concentration was not significantly different between green forage and silages of orchardgrass. The reduction of total tocopherols in conserved forages was observed for both α- and γ-tocopherol, but they did not decrease significantly in orchardgrass silages per comparison to green forage. In conclusion, forage conservation induced reduction in carotenoids (although not zeaxanthin and β-cryptoxanthin) and tocopherols contents. However, some compounds are particularly well conserved in orchardgrass silages.

Reference

Chauveau-Duriot, B., Doreau, M., Noziere, P., Graulet, B., 2010. Analytical and Bioanalytical Chemistry 397, 777-790. https://doi.org/10.1007/s00216-010-3594-y

Funding

Funding was from Auvergne-Rhone-Alpes region through optivim project and French National Agency for Research and Technology through CIFRE grant of B.T DIALLO

The use of blueberry powder as a source of quercetin and its effects on inflammation, oxidation, and short-chain fatty acids in outdoor-housed Siberian huskies in Ontario

P.A.L. Kosmal[1], J. Saunders-Blades[2], D. Kostiuk[2], A.K. Shoveller[1]
[1]University of Guelph, Guelph, Canada. [2]Champion Petfoods Holding Inc, Morinville, Canada (pkosmal@uoguelph.ca)

Blueberries are rich in quercetin, a flavonoid polyphenol known to reduce inflammation and oxidative stress. Given that pet foods often follow trends in human nutrition, blueberries are commonly included as antioxidant sources in commercial pet diets. However, due to differences in gastrointestinal physiology and metabolism among mammals, polyphenol bioavailability and health impact must be studied for each species to determine physiological outcomes and optimal doses. The objectives of this research were to determine, via blood sample analysis, (1) the impact of supplementing blueberry powder on inflammatory cytokines, concentration of short-chain fatty acids (SCFA), and total antioxidant power and (2) if supplementing equivalent levels of quercetin (on a molar basis) from blueberry powder and a quercetin extract provides similar health responses in healthy dogs. Twenty-five privately owned Siberian Huskies (12 females; 3 intact, and 13 males; 4 intact) aged 7 ± 3.7y (mean ± SD) were provided the same wash-in diet for four weeks, followed by 1 of 5 dietary treatments (n=5 per treatment): control diet (CN); control diet + high blueberry powder (HB); control diet + high quercetin extract (HQ); control diet + low blueberry powder (LB); control diet + low quercetin extract (LQ) for six weeks. Blood samples were collected via cephalic venipuncture on days 0, 1, 2, 6, 13, 20, 27, 34, and 41 to assess thirteen serum inflammatory cytokines and chemokines, plasma SCFAs, antioxidant status, and quercetin. Inflammatory cytokines and chemokines were assessed using a commercial cytokine magnetic antibody assay kit (Milliplex MAP Canine Cytokine/Chemokine Panel Multiplex Assay). Free SCFAs were assessed by The Metabolomics Innovation Center (University of Alberta, Edmonton, AB, Canada) using a reverse-phase LC-MS/MS custom assay. Data were analyzed using mixed models for repeated measures with treatment, time, and their interaction as fixed and individual dogs as random effects. Significance was considered at $p<0.05$, and Tukey's post-hoc test was performed to compare means. No treatment x time interaction or treatment effects were found for all assessed cytokines and chemokines ($p>0.05$). Although some differences were found between days across all treatment groups, no clear patterns were established. No treatment effects were found for all assessed SCFAs. Plasma acetic acid was greater on day 41 than on days 0, 1, 13, and 27 across all treatment groups ($p<0.05$). Plasma propionic acid was greater on day 41 than on day 13 across all treatment groups ($p<0.05$). Feeding blueberry powder or quercetin did not impact adult dogs' inflammation and fatty acid concentrations based on blood sample analysis. The absence of treatment effects may reflect the health status of the dogs; with biomarkers within normal physiological range, the ability to detect changes from supplementation may have been limited. The final presentation will include results for quercetin and antioxidant status.

Funding

This work was funded by an NSERC Alliance Grant and Mitacs Accelerate in Collaboration with Champion Petfoods. However, this financial support did not influence the findings or conclusions of this study.

Vitamin and mineral supplementation to gestating F0 beef heifers and the impacts on det digestibility, nitrogen balance, and energy partitioning in the F1 female during pregnancy

J.L. Hurlbert[1], K. C. Swanson[1], A.C.B. Menezes[1], K.K. Sedivec[2], C.R. Dahlen[1]
[1]North Dakota State University, Department of Animal Sciences Center for Nutrition and Pregnancy, Fargo, USA. [2]North Dakota State University, Central Grasslands Research Extension Center, Streeter, USA (jennifer.hurlbert@ndsu.edu)

We evaluated the impacts of providing a vitamin and mineral supplement to F0 beef heifers during gestation on diet digestibility, nitrogen balance, and energy partitioning in the F1 offspring during pregnancy. The treatments of the F0 Angus dams were: the basal diet (CON) or the basal diet plus a vitamin and mineral supplement from breeding to calving (VTM). At 14 to 15 months of age, the F1 heifers (CON, *n* = 7; initial body weight [BW] 417 ± 25.3 kg; VTM, *n* = 8; initial BW 450 ± 28.6 kg) were bred via artificial insemination using female-sexed semen and fed a common diet at 1.5% of BW on a dry-matter (DM) basis throughout pregnancy. At approximately the end of each trimester (d 84, 174, and 240) a 5-day digestibility trial was conducted which included collecting fecal and urine spot samples every 15 h. On d 6, heifers were subjected to 4-h indirect calorimetry measurements using headbox calorimeters. Fecal, urine, and gas samples were evaluated to estimate daily excretion, digestibility, and nitrogen and energy balance. Data were analyzed for the main effects of F0 supplementation, F1 stage in pregnancy, and the respective interaction using the MIXED procedure of SAS. Significance was considered at $P \leq 0.05$ and tendencies considered at $0.05 < P \leq 0.10$. The F1 heifers born to VTM dams were heavier throughout gestation and thus had greater ($P \leq 0.03$) DM and gross energy intake, nutrient intake (OM, N, NDF, and ADF) and greater fecal N excretion ($P = 0.03$) during gestation. Further, VTM F1 heifers tended ($P \leq 0.09$) to have greater DM and OM fecal output, and greater ($P \leq 0.09$) total digested NDF, ADF, and OM. Fecal DM output and OM, N, NDF, and ADF fecal excretion increased ($P = 0.01$) with stage in pregnancy. However, apparent total tract digestibility of DM, N, OM, NDF, and ADF decreased ($P \leq 0.02$) as pregnancy progressed, but there were no differences ($P \geq 0.21$) between CON and VTM. Apparent N digested as a percentage of intake also decreased ($P = 0.01$) throughout gestation, but no differences in treatment were observed ($P = 0.88$). Energy losses in urine ($P = 0.01$) and heat ($P = 0.05$) were greater in VTM F1 heifers. Fecal losses and heat production increased ($P \leq 0.01$) with stage in pregnancy while retained energy decreased but remained positive ($P = 0.01$) as gestation progressed. Finally, the ratio of retained energy to metabolizable energy decreased ($P = 0.01$) as gestation progressed, confirming greater energy losses in the later stages of pregnancy. These data suggest that energy utilization became less efficient in the F1 dam as gestation advanced, as supported by observations in decreased retained energy and increased energy losses. Further research is warranted to determine energy accumulation in pregnancy-related tissues throughout gestation and its influence on current recommended energy requirements for beef heifers.

Funding

We acknowledge our funding support from USDA-NIFA-AFRI #2022-67016-36479 as well as Zoetis and ST Genetics for their donation of estrus synchronization and breeding supplies utilized in this effort.

Can plasma parameters reflect protein and lipid growth dynamics in fattening Charolais bulls? Towards individualized nutritional recommendations

G. Cantalapiedra-Hijar[1], S. Lerch[2], E. Jorge-Smeding[1], H. Nguyen-Ba[1]
[1]Université Clermont Auvergne, INRAE, UMR Herbivores, F-63000, Clermont-Ferrand, France. [2]Ruminant Nutrition and Emissions, Agroscope, 1725, Posieux, Switzerland (gonzalo.cantalapiedra@inrae.fr)

Despite individual variability in feed efficiency and growth potential, contemporary young bulls fattened in the same pen are typically fed identical diets. Recent research aims to refine feeding systems by considering individual nutritional needs to enhance efficiency and performance. The mechanistic INRA growth model predicts daily protein and fat deposition in cattle, which are key factors in determining energy and protein requirements. Initially designed for average animals of specific breeds and sexes, the model may also be calibrated for individual animals by adjusting coefficients for protein and lipid synthesis (α, β), degradation (γ, δ), or modulation of metabolizable energy use efficiency (cMEU). These individual coefficients have been found to be significantly correlated with *in vivo* recorded feed efficiency (Cantalapiedra-Hijar et al., 2023), making them crucial parameters for developing model-based precision feeding strategies. However, estimating these coefficients typically requires individual data on feed intake and body composition, which are difficult to measure in practice. Given that blood plasma metabolites reflect nutrient utilization capacity and body composition, this study aims to contribute to the long-term objective of using plasma metabolite profiles as a practical tool for estimating model coefficients, enabling implementation of model-based individualized feeding strategies. Thirty-six Charolais bulls were fattened on grass-silage-based diets, with or without rumen-protected methionine, over an average of 228±9.6 days. Individual daily metabolizable energy intake and fortnightly body weight were recorded. Body composition was estimated from subcutaneous adipocyte biopsies (begin and mid of the fattening period) and from 6th rib dissection at slaughter. Protein and lipid synthesis rates, degradation rates, or cMEU of the INRA growth model were separately adjusted using the optim function in R software to minimize model deviation from estimated kinetics in body composition. Blood plasma samples were obtained at the beginning of the fattening period and analysed for 13 classical biochemical parameters, natural abundance of ^{15}N and ^{13}C ($\delta^{13}C$), and targeted metabolomic (around 300 metabolites quantification). No correlations greater than 0.5 or less than -0.5 were found between plasma parameters and protein synthesis or degradation rate coefficients (highest correlation, $r = 0.49$ for a lysophosphatidylcholine). However, lipid synthesis and degradation rates were positively ($r > 0.5$) and negatively ($r < -0.5$) correlated ($P < 0.05$) with plasma $\delta^{13}C$, total triglycerides, carnosine, oleic and linoleic fatty acids, and 26 triglyceride isomers, respectively. A partial least square regression model demonstrated that lipid synthesis and degradation rates could be predicted (Q2 = 0.57) using three plasma triglycerides isomers [TG(18:0_32:1), TG(18:1_32:0), TG(18:3_36:1)]. In conclusion, our findings suggest that lipid deposition dynamics can be captured by a set of plasma metabolites measured at the beginning of the fattening period, advancing the potential for precision feeding strategies driven by metabolite profiles coupled with mechanistic modelling.

Reference

Cantalapiedra-Hijar, G., Lerch, S. The INRA mechanistic beef growth model captures feed efficiency ranking in Charolais bulls. 74. Annual Meeting of the European Federation of Animal Science, INRAE, Aug 2023, Lyon, France.

Effects of forage energy and protein content on methane emissions in grazing cattle

G. Simanungkalit, J. Lamb, D. Schneider, J. McLachlan, J. Barwick, F. Cowley
University of New England, Armidale, Australia (gsimanu2@une.edu.au)

Enteric methane (CH_4) fermentation is strongly affected by feed quality and quantity. In a grazing environment, cattle rely primarily on forages, with metabolisable energy (ME) and crude protein (CP) being the key nutritional constituents determining the forage quality. This experiment aimed to investigate the effects of ME and CP content of forages on daily CH_4 production (g/day) in grazing steers. Seventy-two 18-month-old Angus × Bos Indicus steers, averaging (±SD) 324 ± 22.8 kg liveweight, were randomly assigned to 4 groups and maintained in replicate paddocks (18 head/paddock) of 10 hectares each for 142 days. A 30 × 30 m yard was established within each paddock, equipped with a walkover weighing unit, a water trough, 3 supplement feeders, and a GreenFeed (GF) unit to spot-sample individual CH_4 production. The GF data were retrieved from 24 steers (6 untreated steers per paddock), as the remaining 48 were administered a CH_4 inhibitor. Each paddock had been sown with forage oats and annual ryegrass 3 months before commencing the experiment. Pasture sampling was conducted on Days 8 (Cut 1), 51 (Cut 2), and Day 133 (Cut 3) by cutting the pasture within a 50 cm^2 quadrat at 12 randomly selected points in each paddock. Pasture collected at each sample point was bagged and oven-dried at 70 °C for 7 days before being weighed and ground through a 1 mm sieve. The 12 samples from each paddock were then bulked for analysis. The ME was calculated using an equation of ME = 0.203 × dry organic matter digestibility (%) – 3.001, whereas the CP analysis was determined via the Dumas combustion method. The average ME (MJ/kg DM) and CP (%) were 12.6 and 5.2 (Cut 1), 9.1 and 3.8 (Cut 2), and 7 and 3.4 (Cut 3), respectively. Daily CH_4 production data from the GF units were collated based on pasture sampling days (±7 days). The data was analysed using a mixed-effect model with ME, CP, and Paddock as fixed factors and EID within Paddock as a random factor. There was a significant effect of varying CP levels on CH_4 production ($P < 0.001$). A 1-unit increase in CP was associated with a decrease of 15.15 g/day of CH_4 production, whilst a 1-unit increase in ME tended to increase CH_4 production by 3.18 g/day ($P = 0.063$). No significant differences in CH_4 production were observed among paddocks ($P > 0.05$). The random effects revealed substantial variation both between and within individual animals, with standard deviations of 23.6 and 41.8, respectively. Although the levels of ME and CP significantly affected CH_4 production, the change was biologically minimal and prone to individual variation, suggesting that other factors might contribute to modulating CH_4 production in grazing situations.

Funding
This experiment was funded by GrainCorp and the Commonwealth Department of CCEEW

Influence of low phosphorus components in concentrate feed on the protein supply and phosphorus balance of high-yielding dairy cows

M.O. Otten[1,2,3], S.B. Braam[3], G.J. Janknecht[3], J.D. Denißen[3,4], C.B. Böttger[3]
[1]Faculty of Organic Agriculture, University of Kassel, Kassel, Germany. [2]Department of Animal Nutrition, Faculty of Agricultural Sciences and Landscape Architecture, Hochschule Osnabrück, Osnabrück, Germany. [3]Chamber of Agriculture of North Rhine-Westphalia, Kleve, Germany. [4] Agravis Raiffeisen AG, Münster, Germany (martin.otten@lwk.nrw.de)

In Germany, due to a trend towards genetically modified organism-free feeding, rapeseed meal is increasingly used instead of soybean meal. The high phosphorus content in rapeseed meal leads to phosphorus levels exceeding the requirements, surpassing 4.0 g/kg dry matter (DM), when used in dairy cow rations. The excess phosphorus intake is excreted via manure, which is applied to arable land and may leach into the groundwater, contributing to eutrophication. Two trials were conducted in North Rhine-Westphalia, Germany, in 2018 and 2019, each involving 2 × 24 dairy cows. The early-lactation dairy cows, approximately 60 days in milk, were grouped homogeneously based on milk yield, parity, days in milk and body weight. Primiparous cows accounted for 33% in Trial I and 13% in Trial II. The aim of both experiments was to reduce the phosphorus content from Control group in Trial I and Trial II (CON: 4.5; 4.6 g/kg DM) to the low phosphorus group (LP: 3.8; 3.9 g/kg DM) by adjusting the components of the concentrate feed. The phosphorus minimization primarily relied on reducing the amount of rapeseed meal, adding feed urea, and replacing cereal flours with sugar beet pulp in the compound feed. Individual milk yields and dry matter intakes were recorded daily, and milk composition was analyzed weekly. Phosphorus balances were calculated based on the recommendations of the Society for Nutrition Physiology (GfE, 2023). For statistical analyses, the SAS MIXED procedure was used including the fixed effects group, lactation number and lactation curve within lactation number. There were no differences in DM intake between CON and LP group in Trial l (21.1 vs. 20.7 kg, $p = 0.6991$) and Trial II (21.1 vs. 21.7 kg, $p = 0.2144$). In Trial I, the altered concentrate composition did not affect the supply of small intestinal digestible protein (sidP) (2273 vs. 2212 g, $p = 0.6649$), whereas in Trial II, the LP group showed higher sidP supply (2140 vs. 2329 g, $p = 0.0087$). The daily phosphorus intake in LP was reduced by 16% in Trial I (94.6 vs. 79.4 g, $p = 0{,}002$) and by 14% in Trial II (98.1 vs. 84.5 g, $p < 0.001$). No significant differences were found in daily milk yield, weekly milk components (fat, protein, lactose) and energy-corrected milk in Trial I (33.0 vs. 32.5 kg, $p = 0.5493$) and Trial II (36.3 vs. 35.5 kg, $p = 0.4784$). The phosphorus balance according to the GfE (2023) were reduced from 26.9 to 13.1 g/day in Trial I ($p < 0.001$) and from 28.2 to 15.5 g/day in Trial II ($p < 0.001$) by feeding low-phosphorus concentrate feed. These results suggest additional potential for reducing phosphorus excretion through dietary management strategies.

Quantifying the in-vivo pre-cecal digestibility of protein in horses

R.B. Jensen[1,2], E.F. Gjestvang[3], A. Larsson[4], C. Müller[5]
[1]Aarhus University, Tjele, Denmark. [2]Norwegian University of Life Sciences, Ås, Norway. [3]Felleskjøpet Fôrutvikling AS, Trondheim, Norway. [4]Lantmännen Krafft AB, Malmö, Sweden. [5]Swedish University of Agricultural Sciences, Uppsala, Sweden (rbj@anivet.au.dk)

Protein digestion in horses is an area that has received little attention despite its importance for equine health, performance, and sustainable feeding. Current feed evaluation systems do not reflect actual protein digestibility and fundamental knowledge on protein digestion in horses needed to meet today's challenges regarding sustainable feed production. The aim of the present study was to quantify the pre-cecal protein digestibility of eight diverse feedstuffs used for horses. Three cecum cannulated horses fed a hay diet were used to quantify the pre-cecal crude protein digestibility using the mobile bag technique (MBT). The feeds tested were apple pulp, gras pulp, linseeds, rapeseed cake, faba beans, peas, potato protein, and oat protein concentrate. The experiment was approved by the Norwegian Food Safety Authority. On 4 occasions, 20 mobile bags (1x1x12 cm, pore size of 15 µm, filled with 0.5 g of feed) were placed in the stomach of the horses (2 feeds x 10 bags) prior to the morning feeding. The bags contained a small metal piece and a magnet was placed in the cecum of the horses to capture the bags after they had passed through the stomach and small intestine. The magnet was checked every hour for 9 hours. Mobile bags found on the magnet were carefully removed, washed and stored at -20 degrees C for later analysis. Then bags were thawed, washed, dried at 45 degrees C, and weighed. Thereafter, bags from each horse and each feed were pooled and send for chemical analysis. The digestibility of crude protein (dCP) was quantified from the residue in the bags and the protein content of the feeds and the residue. The results showed that the dCP of the feeds varied, and feeds were ranked (from lowest to highest dCP): apple pulp (28.2%), gras pulp (75.5%), linseeds (82.4%), rapeseed cake (89.8%), faba beans (91.4%), peas (93.0%), potato protein (94,2%), and oat protein concentrate (94.2%). In the German feeding recommendations for horses (Coenen et al., 2011), the protein value of feeds for horses are expressed as the pre-cecal digestible crude protein (pcdCP) meausred by chemical analysis. Results from this study will be analysed according to the German system, and correlation analysis will be made. Furthermore, the lowest dCP values were found for the more fibre-rich feeds and more research should focus on the protein digestibility of forages, which make up the majority of the ration for horses.

Reference

Coenen, M., Kienzle, E, Vervuert, I., Zeyner, A., 2011. Journal of Equine Veterinary Science 31, 219-229. https://doi.org/10.1016/j.jevs.2011.03.204

Funding

Lantmännen Research Foundation has funded the project "Sustainable and locally produced protein and energy feeds for horses", and results presented in this originate from this project.

Effects of reduced concentrate feeding on energy metabolism and milk production in early lactation dairy cows

N. Rummukainen[1], P. Rissanen[1], S.E. Räisänen[1,2], S. Salin[1], A. Halmemies-Beauchet-Filleau[1], M. Lamminen[1], A. Vanhatalo[1], T. Kokkonen[1]
[1]Department of Agricultural Sciences, Faculty of Agriculture and Forestry, University of Helsinki, Helsinki, Finland. [2]Department of Animal and Veterinary Sciences, Research Centre Foulum, Aarhus University, Tjele, Denmark (netta.rummukainen@helsinki.fi)

We studied the effects of two different dietary concentrate proportions, 37.5% and 50% of diet dry matter (DM), on milk yield (MY), feed intake and metabolism of dairy cows. The experimental design was a randomized block-design, starting from calving. In total, 30 Finnish Ayrshire cows of second parity or greater were assigned to pairs based on parity, expected calving date, milk production data from the beginning of previous lactation and body weight. The amount of concentrate was adjusted from the milking robot and both groups had ad libitum access to the same partial mixed ration (PMR) consisting of grass silage and cereal concentrate. Cows fed a high-concentrate diet received 8 kg/d of concentrate from the milking robot and cows with low-concentrate diet 2 kg/d. Feed intake and milk yield were recorded daily during the first two months of lactation and milk samples were collected once per week during weeks 1,2,3,4,6 and 8, rumen fluid samples once per week during weeks 1,3 and 6 and blood samples once per week during weeks 1,2 and 3 after calving. Fecal spot samples were collected on three consecutive days on the sixth week of lactation. Feed samples from silage, robot concentrate, and concentrate used in PMR were collected weekly. Data were analyzed with repeated measures ANOVA. The model included the fixed effect of treatment, week, treatment x week and the random effect of pair and week x pair. Digestibility of the grass silage was moderate, average D-value (concentration of digestible organic matter in DM) was 661g/kg DM. There were no differences in total DM intake between dietary treatments, while cows fed a low-concentrate diet had greater PMR intake. Cows fed with less concentrate had 4.1 kg/d lower ($p < 0.01$) MY and 2.4 kg/d lower ($p < 0.10$) energy-corrected milk yield. Dietary concentrate proportion had no effect on milk composition, although the fat and protein contents were numerically greater [protein 36.6 vs 35.5 g/kg (SEM 0.66) and fat 45.9 vs 43.7 g/kg (SEM 1.60)] when cows received less concentrate. There were no differences between the two groups in daily rumination time, body weight or plasma concentrations of non-esterified fatty acids, beta-hydroxybutyrate or glucose. Cows fed a high-concentrate diet had lower ($p < 0.01$) fecal pH and tended ($p < 0.10$) to have lower reticular pH. Despite moderate digestibility of the silage, cows fed with less concentrate did not have more signs of metabolic problems, such as ketosis, when compared to cows that were fed with high concentrate proportion. Based on these preliminary results, reducing concentrate feeding decreased MY substantially. Additional analyses will be conducted to assess the dietary effects on cow metabolism and welfare, as well as the economic and environmental impacts.

Funding

Project received funding from the Ministry of Agriculture and Forestry of Finland, Helsinki, Finland.

Manganese as an intrinsic marker for total tract digestibility in dairy cows

M.J. Brask, E. Hvas, M. Larsen, N.P. Hansen, S. Lashkari, C.F. Børsting, M.R. Weisbjerg, N.B. Kristensen
Department of Animal and Veterinary Sciences, Aarhus University, Tjele, Denmark (maike.brask@anivet.au.dk)

The validity, precision and accuracy of digestibility measurements based on markers depends on the marker being chemically inert, flow dynamics of the marker relative to the feed fractions in question, application methods (internal, mixed into the diet, pulse dose, infusion), basal ration or sampling interval. Continuous infusion of CrEDTA or $YbCl_3 \cdot 6H_2O$ via the rumen cannula are well consolidated markers, but also costly, work intensive and require invasive animal methods. For measuring fecal dry matter (DM) flow in order to calculate total tract DM digestibility, for larger groups of cows, intrinsic markers might be more cost effective and minimize risks of undesirable host, feed, and marker interactions. Manganese (Mn) is a trace element with very low absorption rates in dairy cows (Daniel et al., 2023) and hence has the potential to be used as an internal marker for total tract digestibility. In the underlying trial, Mn was compared against infused markers CrEDTA and $YbCl_3 \cdot 6H_2O$ in 4x4 Latin square with four multi-fistulated lactating cows. Cows were milked and fed twice daily. They received one of four diets including high or low proportions of maize and grass silage and one of two different fat sources. None of the diets were supplemented with Mn from mineral premix. The TMRs contained between 70 and 90 mg/kg DM of Mn from feedstuffs. CrEDTA and $YbCl_3 \cdot 6H_2O$ were dissolved in water with a target concentration of 2,84 % CrEDTA and 0,55 % $YbCl_3 \cdot 6H_2O$ and infused continuously via the rumen cannula, starting 72 hours before first sampling. Over a four-day period, 8 fecal samples were taken every 3rd hour of the day to represent diurnal variation. All analyses were made in a composite sample. Results were analyzed using the ggplot package in R with a linear regression between the average of fecal DM flow measured by CrEDTA and $YbCl_3 \cdot 6H_2O$, against fecal DM flow measured by Mn. Calculations were corrected for the assumption that 2.3 % of ingested Mn is not recovered in feces (Weiss and Socha, 2005). The regression analysis of Cr and Yb average against Mn resulted in R^2 values of 0.75. Despite differences in level of fecal output, both approaches ranked the fecal output and digestibility of the experimental diets in the same order and would therefore lead to the same conclusions when using Mn as the sole marker. In conclusion, Mn has the potential for being an inexpensive intrinsic digestibility marker. It is relevant for use in combination with diets supplemented with Mn free mineral feed, especially for non-invasive experiments with larger groups of intact cows, which are part of the food chain. Correction for absorption rates, especially with Mn supplemented pre-mix, should be further investigated.

References

Daniel, J.P., Brugger, D., van der Drift, S., van der Merwe, D., Kendall, N., Windisch, W., Doelman, J., Martín-Tereso, J., 2023. The Journal of Nutrition 153, 1008-1018. https://doi.org/10.1016/j.tjnut.2023.02.022

Weiss, W.P., Socha, M.T., 2005. Journal of Dairy Science 88, 2517-2523. https://doi.org/10.3168/jds.S0022-0302(05)72929-5

Linear mixed model and random forest algorithm outperformed current estimation method in predicting nitrogen utilization efficiency of broiler chickens

W. Dou, T.M. Lange, W. Siegert
University of Göttingen, Göttingen, Germany (wolfgang.siegert@uni-goettingen.de)

The experimental determination of nitrogen utilization efficiency (nitrogen (N) accretion/N intake; NUE) of broiler chickens by whole-body analysis or excreta collection is restrained due to regulations and effort. A practical and currently widely used alternative is to estimate accreted N assuming a consistent 29 g/kg weight gain (C29) [e.g., 1], which is less accurate. This contribution aimed to develop an improved NUE prediction based on information widely available in broiler rearing. A dataset of 528 data lines was extracted from 90 peer-reviewed publications from 2016 – 2024. This dataset excluded data lines where performance was significantly reduced by an intervention. Two simulations were applied each 1000 times splitting into 70% training and 30% testing data for Monte Carlo cross-validation: (I) random split; (II) split by trial to avoid shared trial effects between the two sets. The C29 approach was compared with a linear mixed model with a random trial effect (LMM) and random forest algorithm (RF). Both models included five predictors: dietary crude protein (CP) and metabolizable energy (ME_N), age, feed intake, and weight gain. The predictor combination producing the highest goodness-of-fit in a full-dataset trial was subsequently applied to the cross-validation. The optimized number of trees was firstly determined and implemented for RF using R packages "optRF" and "randomForest", respectively. In the cross-validation, models were built using the training set and applied to the testing set for prediction. Goodness-of-fit was assessed using the R^2 and RMSE between predicted and observed NUE. Concordance correlation coefficient (CCC) was computed to indicate stability. In the random-split simulation, LMM exhibited the highest goodness-of-fit (R^2 0.795, RMSE 3.6) and CCC (0.88), hence outperforming RF with 1000 trees (R^2 0.631, RMSE 4.9, CCC 0.716; prediction stability 0.9989) and C29 (R^2 0.128, RMSE 12.7, CCC 0.29). Goodness-of-fit of all models was lower in the split-by-trial simulations: The highest R^2 and CCC were from C29 (0.130, 0.26, respectively), with very high RMSE (12.8). LMM was more accurate and stable than RF with 2000 trees (R^2 0.124 vs 0.050; RMSE 7.6 vs 8.1; CCC 0.18 vs 0.10) with prediction stability for RF at 0.9994. All differences of the parameters among models mentioned above were significant ($P<0.05$). This study indicated that the current method estimating NUE based on weight gain can be improved by integrating more information that is commonly accessible in farming practice to produce more accurate predictions. Further advancement in prediction accuracy seems to require less widely available predictors.

Reference

Belloir, P., Méda, B., Lambert, W., Corrent, E., Juin, H., Lessire, M., Tesseraud, S., 2017. Animal 11, 1881-1889. https://doi.org/10.1017/S1751731117000660

Funding

This research was performed without external funding.

Importance of in sacco and mobile bag methods for determination of protein utilization in cattle

M. Rajský, Z. Formelová, Z. Mlyneková, M. Chrenková
National Agricultural and Food Centre - Research Institute for Animal Production Nitra, Lužianky, Slovakia
(matus.rajsky@nppc.sk)

Although the use of live animals, especially those that have undergone surgical procedures, is decreasing in research, they continue to play an important role. These are two discussed perspectives: on one hand, the welfare of animals used in research, and on the other hand, the accuracy of data obtained through alternative *in vitro* methods. At the accredited Laboratory of Physiology of Ruminant Nutrition, we use cows with ruminal and duodenal cannulas, so we compare the results obtained *in vivo* with those obtained using alternative *in vitro* methods. Determination of rumen degradability and intestinal digestibility of protein is a prerequisite to adjust cattle rations within a precision feeding framework, thereby reducing ammonia nitrogen release. Since the increasing productivity of dairy cows requires a reduction in the proportion of protein degraded in the rumen, as well as an increase in the digestibility of proteins transported to the small intestine, monitoring of protein bypass using *in sacco* and *mobile bag* methods led to the development of a self-designed duodenal cannula, including surgical application and its intellectual property protection. Our cannula for determination of intestinal digestibility of nutrients in cattle has been registered by the European Union Intellectual Property Office, as a Registered Community Design in 2023 (No 015036121-0001). The characteristics of the cannula confirm the usefulness of the design for the folowing purposes: low weight not causing traction on tissues, replacement after removal without surgical intervention, possibility of cannula extension during the growth of the animal, which allows its use in cannulated cows without time limitation, even at the age of 15 years and older. When comparing the results of the *in sacco* method and the Cornell Net Carbohydrate and Protein System (CNCPS), we found a correlation between effective degradability of crude protein and soluble fractions in concentrate feeds expressed by the Pearson correlation coefficient r=0.5464 and in forages r=0.6323 (Mlyneková et al. 2023). It was not possible to derive parameters from the CNCPS results that would allow the calculation of effective degradability of crude protein. When comparing the results of intestinal digestibility determined by the *mobile bag* method and the two-step enzymatic method using the Daisy II system, we observed a stronger correlation; however, the *in vitro* results were systematically lower compared to the *in vivo* method. For extracted meals and cakes, we determined r=0.957, for legumes r=0.888, for DDGS r=0.736, and for green forages r=0.629. Due to these differing results and the possibility of providing good living conditions for cannulated cows, we currently also use the *in sacco* and *mobile bag* methods. This opens up opportunity for further partnerships in new research projects.

Reference

Mlyneková, Z., Formelová, Z., Chrenková, M., Rajský, M., Polačiková, M., 2023. Journal of Central European Agriculture 24, 349-357. https://doi.org/10.5513/JCEA01/24.2.3747

Funding

This work was supported by the Slovak Research and Development Agency under the Contract no. APVV-22-0349.

Optimizing feed production processes to increase protein utilization in cattle

Z. Formelová[1], Z. Mlyneková[1], G. Bruggeman[2], S. Baxa[3], M. Rajský[1]
[1]National Agricultural and Food Centre - Research Institute for Animal Production Nitra, Lužianky, Slovakia. [2]Nutrition Sciences N. V., Booiebos, Gent, Belgium. [3]National Agricultural and Food Centre - Food Research Institute, Bratislava, Slovakia (matus.rajsky@nppc.sk)

Rumen degradability and the intestinal digestibility are key parameters in the evaluation of dietary protein. It is well established that improving protein utilization in cattle can be achieved by employing feed processing technologies that protect dietary protein from excessive microbial enzymatic degradation in the rumen. This allows a greater proportion of protein to bypass the rumen and become available for digestion and absorption in the small intestine. Results from the Laboratory of Physiology of Ruminant Nutrition at the National Agricultural and Food Centre, obtained using *in sacco* and *mobile bag* methods, indicate that the effective degradability of crude proteins (EDgCP) varies considerably depending on the characteristics of the feed and the treatment method applied. EDgCP values ranged from as low as 29.69% in encapsulated soybean meal to as high as 90.62% in sunflower cake treated with a different encapsulation method. Encapsulation of extracted soybean meal using fat as a coating reduced EDgCP from 72.3% to 29.69%. When extrusion was used as the processing method, EDgCP was determined to be 40.21%. The intestinal digestibility of soybean meal processed using these methods was determined to be 98.3% for untreated meal, 87.59% for encapsulated, and 99.1% for extruded soybean meal. Extrusion and encapsulation thus had a significant impact on increasing the utilization of soybean proteins. In practice, this means that from a feed ration containing 1000 g of soy crude protein, a dairy cow can utilize in the small intestine 272 g from untreated soybean meal, 593 g from extruded soybean meal, and 616 g from encapsulated soybean meal. On the other hand, it is also important to mention processing methods that did not improve the nutritional value of the feed. For example, coating sunflower cake with whey permeate high in lactose resulted in an undesirable increase in EDgCP from 82.58% to 90.62%. In contrast, extrusion proved to be an effective treatment method, lowering EDgCP from 82.58% to 68.4%. If the treatment does not provide sufficient protection of crude proteins, e.g., through stable bond formation or physical isolation, it may have the opposite effect - increasing their rumen degradability. The key lies in correctly adjusting the type, intensity, and conditions of the treatment. The intestinal digestibility of sunflower cake was found to be 68.96% in untreated form, 57.69% in encapsulated form, and 84.89% in extruded form. The application of *in sacco* and *mobile bag* methods in canulated cows enables the verification of feed efficacy claims made by manufacturers and provides essential data for precision feeding strategies.

Funding

This work was supported by the Slovak Research and Development Agency under the Contract no. APVV-22-0349.

Effects of Cryptosporidium parvum infection on physico-chemical characteristics of the gastrointestinal content and microbiota in neonatal calves

W. Liermann[1], H. Reyer[1], F. Dengler[2], C. Delling[3], L. Bachmann[4], R. Ulrich[3], C. Helm[3], H.M. Hammon[1]
[1]Research Institute for Farm Animal Biology (FBN), Dummerstorf, Germany. [2]University of Hohenheim, Stuttgart, Germany. [3]Leipzig University, Leipzig, Germany. [4]University of Applied Science Neubrandenburg, Neubrandenburg, Germany (liermann@fbn-dummerstorf.de)

Cryptosporidium parvum is a major pathogen causing diarrhoea in calves in the first weeks of life (Windeyer et al., 2014). The establishment and maintenance of a healthy and functional gastrointestinal tract is important for the whole organism and is largely related to the development of a diverse and beneficial microbial community. The present study aimed to investigate the influences of a *C. parvum* infection on physico-chemical characteristics and microbial community of the gastrointestinal content in the abomasum, jejunum and colon of 8-day old calves. Therefore, male Holstein-calves infected on day 2 and slaughtered on day 8 of life were compared to uninfected control calves (n = 5 per group). Dry matter content and lactate were analysed by an automatic moisture and an automatic clinical chemistry analyser, volatile fatty acids by gas chromatography and the microbiota by targeted sequencing of the microbial 16S rRNA gene. Statistical analyses of the microbiota were done by DESeq2 package of R. MIXED procedure of SAS were used to reveal differences between the diversity indices, dry matter content, fatty acids and lactate. Fixed effects were group and gastrointestinal segment as well as their interactions. Pooled Least Squares Means (LSMeans) of the dry matter content were lower in gastrointestinal tract of infected animals. Acetate concentrations were lower and lactate concentrations tended to be lower in colon of infected than non-infected calves. The Shannon and Simpsons microbial diversity indices decreased from abomasum to colon. Pooled LSMeans of Shannon diversity index were lower and of Simpsons diversity index tended to be lower in infected calves compared to non-infected calves. In abomasum of infected calves no *Butiricimonas* was detected, but in control animals this genus was present. Furthermore, a lower abundance of *Sphingobacterium* was detected in the abomasum of infected compared to control calves. Infected calves showed a lower abundance of *Bifidobacterium* than non-infected calves but a higher abundance of *Clostridium sensu strictu 1* in jejunum and colon. Further, the abundance of Veillonellaceae (unclassified) was higher and of *Sphingobacterium* was lower in jejunum of infected compared to non-infected calves. In conclusion, although *C. parvum* predominantly colonize ileal segment the present study indicates that *C. parvum* infection induces adverse modulations in the gastrointestinal content and the microbial community in the whole gastrointestinal tract. Because of the importance of the early development for a beneficial microbiota and possible priming effects, these alterations might play a key role for the further gastrointestinal development and metabolism of growing calves.

Reference

Windeyer, M.C., Leslie, K.E., Godden, S.M., Hodgins, D.C., Lissemore, K.D., LeBlanc, S.J., 2014. Preventive Veterinary Medicine 113, 231-240. https://doi.org/10.1016/j.prevetmed.2013.10.019

Evaluating the suitability of the bovine hepatocyte cell line BFH12 as an in vitro model for liver metabolism in dairy cows

L. Dietz[1], R. Cermak[1], W. Grünberg[2], M.R. Wilkens[3]
[1]Institute of Veterinary Physiology, Leipzig University, Leipzig, Germany. [2]Clinic for Ruminants and Herd Health Management, Justus-Liebig-University of Giessen, Giessen, Germany. [3]Institute of Animal Nutrition, Nutrition Diseases and Dietetics, Leipzig University, Leipzig, Germany (mirja.wilkens@uni-leipzig.de)

The objective of this preliminary study was to investigate if the immortalized cell line BFH12 derived from bovine fetal hepatocytes is a suitable tool to address the challenge of intermediary metabolism of dairy cows during negative energy balance in early lactation. To this end, we compared gene expression changes determined in liver biopsies collected from dairy cows 2 to 4 weeks (wk) before and 2 wk after parturition (p.p.) with the gene expression pattern of BFH12 incubated under varying conditions. The target genes chosen were associated with the somatotropic axis (growth hormone receptor 1A, GHR1A, insulin-like growth factor 1, IGF1), glycolysis (phosphofructokinase 1, PFK1), gluconeogenesis (pyruvate carboxylase, PC, phosphoenolpyruvate carboxykinase, PCK2), ketogenesis (3-hydroxy-3-methylglutaryl-CoA synthase 2, HMGCS2), fatty acid oxidation (Carnitine palmitoyltransferase I, CPT1A, acyl-CoA oxidase 1, ACOX1), and fatty acid synthesis (acetyl-CoA carboxylase, ACC1, fatty acid synthase, FASN, stearoyl-CoA 9-desaturase, SCD1). In the liver biopsies (N = 5), gene expression of GHR1A and IGF1 determined via standard quantitative RT-PCR was downregulated (student t-test for paired observations, $P < 0.01$), while ACOX1 was upregulated p.p. ($P = 0.05$). CPT1A, HMGCS2, PC, PFK1 and ACC1 expression showed substantial variances p.p. probably related to individual differences in energy balance. In a first step, BFH12 cells (N = 3) were cultivated with 11.5 mM glucose, 100 nM dexamethasone and 0.2 U/mL insulin (+DEX+INS) according to the protocol from the literature [Gleich et al, 2016]. When BFH12 cells were cultivated for 72 h either without dexamethasone (-DEX), without insulin (-INS), or without both (-DEX-INS), GHR1A expression was decreased in -DEX and -DEX-INS (mixed model for the fixed effects of DEX, INS and the interaction, $P < 0.05$), CPT1A expression was decreased in -DEX-INS ($P < 0.05$), PCK2 in -INS ($P < 0.05$), and PFK1 in -DEX-INS ($P < 0.05$). In a second step, we compared +DEX+INS and -DEX-INS BFH12 cultivated with either 11.5 mM, 3.0 mM or 1.5 mM glucose for 24 h (N = 3). A mixed model (media and concentrations of glucose) confirmed the effect of -DEX-INS on the expression of GHR1A ($P < 0.05$) and a trend for altered CPT1A expression ($P < 0.1$) and revealed a trend for a decreasing effect of low glucose concentrations on GHR1A in -DEX-INS cultivated cells ($P = 0.08$) and SCD1 irrespective of DEX and INS ($P = 0.10$). IGF1 and HMGCS2 were not expressed in BFH12 cells. These results are in line with the observation that BFH12 cells accumulate triglycerides when cultivated with oleic acid [Reichelt et al., 2022]. It may be concluded that BFH12 cells might be a suitable model for hepatic lipogenesis but not for the somatotropic axis, carbohydrate metabolism and ketogenesis.

References

Gleich, A., Kaiser, B., Schumann, J., Fuhrmann, H., 2016. Cell and Tissue Research 52, 662-672. https://doi.org/10.1007/s11626-016-0018-0

Reichelt, K., Niebisch, A.M., Kacza, J., Schoeniger, A., Fuhrmann, H., 2022. Frontiers in Veterinary Science 9, 840202. https://doi.org/10.3389/fvets.2022.840202

Supplemental protein concentrate to postpartum dairy cows increases milk production with positive carry-over effects

S.E. Räisänen, T. Michou, M.R. Weisbjerg, M. Larsen
Department of Animal and Veterinary Sciences, Aarhus University, Tjele, Denmark
(susanna.raisanen@anivet.au.dk)

Previous studies have demonstrated that abomasal infusion of casein to postpartum transition dairy cows can increase milk yield (MY) by around 7 kg/d for the first 4 weeks in milk (Larsen et al., 2014), but longer-term carry-over effects have been difficult to obtain with practical diets. This on-going pilot study aimed to assess the carry-over effect of short-term protein supplementation in early lactation on milk production of dairy cows. Twenty-five Danish Holstein cows were included in the experiment, of which 10 calved into second and 15 into third or fourth lactation. Within parity group, cows were assigned to one of two treatments: 1) a standard lactation diet (CON; 16.7% CP of DM, 32.8% NDF, and 17.0% starch) and 2) CON + protein concentrate [BOOST (42.3% CP, 7.9% NDF, 12.2% starch); 4 kg/d from 1 to 7 DIM, then reduced by 200 g/d until 27 DIM]. The cows had ad libitum access to the basal diet in a Roughage Intake Control system (RIC; Insentec BV). The protein concentrate was provided in a separate RIC bin and allowance was adjusted according to DIM. Daily intake of basal diet and protein concentrate, and MY were recorded. Data were analyzed using the *lmer* function in R with treatment, DIM and their interaction, and parity as fixed and cow as random effects. Daily intake of protein concentrate from 1 to 7 DIM averaged 3.24 kg (SEM = 0.499), and decreased on average 204 g/d from 8 to 27 DIM. Milk yield was 7.1 kg/d greater ($P \leq 0.01$; SEM = 1.96; n = 13 cows/treatment) for BOOST vs. CON cows on 1 to 7 DIM. During the gradual decrease of BOOST until 27 DIM, the difference in MY persisted, being on average 47.0 vs. 39.0 kg/d ($P \leq 0.001$; SEM = 1.98; n = 13). After discontinuation of BOOST, from 28 to 45 DIM, MY continued to be around 5.7 kg/d greater ($P \leq 0.02$; SEM = 2.09; n = 8 to 11) for the BOOST cows. Total dry matter intake did not differ between treatments ($P = 0.58$) and averaged 18.2 kg/d (SEM = 1.46) from 1 to 7 DIM, 22.2 kg/d (SEM = 1.46) from 8 to 27 DIM, and 24.9 kg/d (SEM = 1.71) from 28 to 45 DIM. These preliminary results indicate that providing a metabolizable protein boost as a separate concentrate feed in the very first days of lactation can improve milk production with positive carry-over effects into at least the first 6 weeks of lactation. This on-going study will further test the effects of BOOST on milk composition, nitrogen utilization, and metabolic and physiological parameters throughout lactation.

Reference

Larsen, M., Lapierre, H., Kristensen, N.B., 2014. Journal of Dairy Science 97, 5608-5622. https://doi.org/10.3168/jds.2013-7247

Effect of including resistant potato starch in transition diets for sows on litter gain, colostrum intake and fecal score of sows

E.M. Christensen[1], A.L. Voergaard[2], A.V. Strathe[1]
[1]University of Copenhagen, Copenhagen, Denmark. [2]KMC, Brande, Denmark (avst@sund.ku.dk)

Colostrum intake is crucial for survival and growth of the newborn piglet. The aim of this study was to test the effect of including resistant potato starch in transition diets for sows on early piglet growth, colostrum intake and fecal score of sows. Twenty Yorkshire sows (Topigs NorSvin) were allotted to either a control diet (CON) or the control diet including resistant potato starch (STAR). The standard diet was based on wheat, barley, soybean meal and sugar beet pellets. Sows fed the STAR diet were given 325 g of resistant potato starch (ReStarch, KMC, Denmark) as top-dressing on their meals two times per day from one week prior to expected farrowing. Weight of the individual piglets was registered at birth, 12 h and 24 h and gain was calculated. The colostrum intake for the first 24 h after birth was estimated using equation by Theil et al. (2014). Within one hour of birth of the first piglet a colostrum sample was obtained by hand milking the sow. A Brix measurement was done immediately after sampling. Fecal score of sows was registered according to Oliviero et al. (2009) two times per day at insertion into the farrowing unit, the day before expected farrowing and the day after farrowing. The data was analysed using a statistical model including dietary treatment and parity as fixed effects and batch of sows as a random effect. The preliminary results of the study showed that the totalborn (18.1 vs. 19.5; P=0.862) and litter birth weight (22.1 vs. 23.1 kg; P=0.199) for CON and STAR sows, respectively, was unaffected by dietary treatment. Litter gain for the first 12h (P=0.195) and 24h (P=0.431) was 57.8 and 52.7 g for CON and 21.8 and 30.7 g for STAR sows, respectively. Total colostrum intake for the litter (P=0.981) and the average per piglet (P=0.422) for the first 24 h was 5686 g and 400g for CON and 5352 g and 389 g for STAR sows, respectively. The Brix value of the colostrum was similar for CON and STAR sows, respectively (25.6 vs. 25.4; P=0.922). Fecal scores were similar at insertion (P=0.268), whereas the score was higher in STAR sows compared to CON sows 1 d before expected farrowing (2.7 vs. 1.8; P<0.01) and 1 d after farrowing (2.3 vs. 1.4; P=0.096) there was a tendency for higher score in STAR sows. In conclusion, RPS did not affect colostrum production and piglet growth during the first 24 hours. Resistant potato starch did result in a higher fecal score both before and after farrowing, which could indicate that RPS can help prevent constipation.

References

Oliviero, C., Kokkonen, T., Heinonen, M., Sankari, S., Peltoniemi, O., 2009. Research in Veterinary Science 86, 314-319. https://doi.org/10.1016/j.rvsc.2008.07.007
Theil, P.K., Flummer, C., Hurley, W.L., Kristensen, N.B., Labouriau, R.L., Sørensen, M.T., 2014. Journal of Animal Science 92, 5507-5519. https://doi.org/10.2527/jas.2014-7841

Funding

The study was funded by the PigWeb Project (Horizon 2020)

Omics-powered extrapolation of changes in energy and protein metabolism in neonatal calves during Cryptosporidium parvum infection

W. Liermann[1], A. Veshkini[2,1], F. Dengler[3], L. Bachmann[4], R. Ulrich[5], C. Delling[6], D. Viala[2,7], C. Kühn[8,9], M. Bonnet[2], H.M. Hammon[1]

[1]Research Institute for Farm Animal Biology (FBN), Dummerstorf, Germany. [2]INRAE, Université Clermont Auvergne, VetAgro Sup, UMR Herbivores, Saint-Genès-Champanelle, France. [3]Institute of Animal Sciences, University of Hohenheim, Stuttgart, Germany. [4]Faculty of Agriculture and Food Science, University of Applied Science Neubrandenburg, Neubrandenburg, Germany. [5]Institute for Veterinary Pathology, Leipzig University, Leipzig, Germany. [6]Institute of Veterinary Parasitology, Leipzig University, Leipzig, Germany. [7]INRAE, Metabolism Exploration Platform, proteomic component (PFEMcp), Saint-Genès-Champanelle, France. [8]Friedrich-Loeffler-Institute, Greifswald-Insel Riems, Germany. [9]Agricultural and Environmental Faculty, University of Rostock, Rostock, Germany (hammon@fbn-dummerstorf.de)

Cryptosporidium parvum (*C. parvum*) infection in neonatal calves induces profound host metabolic changes, driven by the parasite's extensive scavenging of host glucose and amino acids (AAs), but the consequences have not yet been fully understood. We used an integrated multi-omics approach encompassing transcriptomics, proteomics, and metabolomics, to follow up a systems-level view of calves metabolic reprogramming following *C. parvum* infection. Ten male Holstein-Friesian calves were assigned to orally receive either: 1) pure water as a control (CTRL, N = 5) or 2) *C. parvum* oocysts to induce infectious diarrhea (INF, N = 5) on d 1 of life. On day 7 post-infection, calves were euthanized via exsanguination, and samples were collected from the jejunal tissue, intestinal contents, and plasma. Jejunal tissue was subjected to transcriptomics and proteomics profiling, while metabolomics analyses were performed on intestinal contents and plasma samples. The resulting multi-omics datasets were integrated to generate a comprehensive database of differentially abundant biomolecules, enabling system-level bioinformatics analysis of host metabolic responses to *C. parvum* infection. Gene names, but not proteins, were italicized. The metabolome results revealed systemic reductions in plasma glucose, cholesterol, HDL, and LDL levels, confirming shifts in host energy and lipid metabolism. In the small intestinal epithelial cells, the deregulation of glycolytic enzymes (*PFKM*, *PFKP*, *PFKl*) and mitochondrial electron transport chain components (*COX7A1*, UQCRB), reflects a disruption of glucose metabolism and mitochondrial function, leading to compromised flux through glycolysis, the TCA cycle, and oxidative phosphorylation. On the other side, a marked upregulation of peroxisomal and mitochondrial fatty acid oxidation enzymes (ACOX1, *ABCD1*, *CROT*, *ETFDH*) facilitates the generation of lipid-derived acetyl-CoA, which is rerouted into ketogenesis (via enzymes such as *ACAT1* and *HMGCS2*) and supports cholesterol biosynthesis through activation of the SREBP-regulated pathway (e.g., *HMGCR*, *FDFT1*, *SQLE*). In parallel, enhanced expression of *BDH1* and *OXCT1* indicates increased peripheral utilization of ketone bodies to support energy production. Simultaneously, we observed altered concentrations of key AAs in the intestinal contents, particularly arginine and tryptophan and ketogenic AA, which may partly provide additional ATP but also other metabolic intermediates. Concurrent upregulation of proteins involved in proteostasis [cellular protein turnover], oxidative response, and translation control (*HSPA1A*, EIF2AK2, VCP, SOD1) suggests a broader stress-adaptive response involving protein metabolism. Altogether, these findings provide a better understanding of how neonatal calves shift from carbohydrate-based to lipid-based energy metabolism, alongside AAs engagements, to maintain energy and protein homeostasis during and after infection with *C. parvum*.

Effects of the use of the microalgae *Chlorella vulgaris* as a dietary supplement in the rearing of Atlantic salmon in land-based aquaculture

J. Mueller[1,2], D.R. van Muilekom[3], J. Ehlers[2], M. Suhr[4], S.C. Hornburg[4], C. Bang[5], M. Wilkes[5], T. Schultheiß[6], E. Maser[6], A. Rebl[3], T. Goldammer[3,7], H. Seibel[2], C. Schulz[1,2]
[1]Institute of Animal Breeding and Husbandry, Department for Marine Aquaculture, Kiel University, Kiel, Germany. [2]Aquaculture and Aquatic Resources, Fraunhofer Research Institution for Individualized and Cell-Based Medical Engineering IMTE, Büsum, Germany. [3]Research Institute for Farm Animal Biology, Fish Genetics, Dummerstorf, Germany. [4]Institute of Animal Nutrition and Physiology, Kiel University, Kiel, Germany. [5]Institute of Clinical Molecular Biology, Kiel University, Kiel, Germany. [6]Institute of Toxicology and Pharmacology for Natural Scientists, Faculty of Medicine, Kiel University, Kiel, Germany. [7]Molecular Biology and Fish Genetics, Faculty of Agriculture and Environmental Sciences, University of Rostock, Rostock, Germany (tomgoldammer@fbn-dummerstorf.de)

Atlantic salmon (*Salmo salar*) is one of the most valued fish species for human consumption, with a global production of 2.7 M tons in 2020. However, sea water production is associated with compromised health and high losses, which is why land-based recirculation systems (RAS) are increasingly being used. This in turn poses challenges, as a number of stress factors can negatively affect the health of salmon in RAS. We investigated whether microalgae-enriched functional feeds can improve the health and growth performance of Atlantic salmon in RAS and allow the fish to cope more effectively with stress. Based on our previous data (Mueller et al., 2023), this study aimed to investigate the use of *Chlorella vulgaris* as a functional feed additive in diets for Atlantic salmon (Mueller et al., 2024). More specifically, we hypothesized that the inclusion rate (2%, CV2 vs. 14%, CV14) and mode of application (daily vs. weekly, CV14w) of *Chlorella vulgaris* fed to Atlantic salmon would influence growth performance and the microbiota of the digesta as well as intestinal mucosa. Furthermore, feeding the functional enriched diets was hypothesized to counteract stress- and immune-related effects caused by an acute treatment with peracetic acid (CH_3CO_3H; PAA, a commonly used disinfectant in RAS) as an oxidative stressor for one hour. While CV14 increased feed conversion (FCR) significantly, feeding the diets CV2 and CV14w improved protein retention efficiency. CV14 significantly modulated beta-diversity in the intestinal digesta and mucosa, but this effect was already visible in fish fed CV2. Feeding CV14 and, to a lesser degree, CV2 increased the relative abundances of the microbiota *Paenarthrobacter* and *Trichococcus* in the digesta and mucosa, which are able to metabolize complex carbohydrates. However, the same diets reduced the abundance of the lactic acid bacteria *Lactobacillus* and *Weissella* in the digesta and *Floricoccus* in the mucosa. Peracetic acid exposure induced systemic stress (increase in plasma glucose and cortisol) and a local immune response in the gill, with the most prominent upregulation of several immune- and stress-regulated genes (*clra, cebpb, marco, tnfrsf14, ikba, c1ql2, drtp1*) 18 h after exposure in fish fed the control diet. Fish receiving CV14 once a week showed a reduced transcriptional response to PAA exposure. Overall, this study revealed that the health of Atlantic salmon can be influenced by dietary application of *Chlorella vulgaris* where the intestinal microbiota was most influenced by feeding 14% *Chlorella vulgaris* daily, whereas weekly feeding seemed to increase stress resilience to PAA.

References

Mueller, et al., 2023. Frontiers in Marine Sciences 10. https://doi.org/10.3389/fmars.2023.1273614
Mueller, et al., 2024. Scientific Reports 14(1), 23674. https://doi:10.1038/s41598-024-72531-8

Funding

The research proposal BioFiA "Bioindicators for the evaluation of fish farming, health and product quality in different aquaculture systems" (projects: 031B09151E1, 031B09151E2, 031B09151E8) was funded by BMBF (Germany). Microbiome sequencing was supported by DFG Excellence Cluster 2167 "Precision Medicine in Chronic Inflammation" (PMI) and DFG Research Unit 5042 “miTarget”.

Author index

(Underlined pages indicate that the author was presenting the work)

Research Institute for
Farm Animal Biology

Wilhelm-Stahl-Allee 2, 18196 Dummerstorf, Germany

https://www.fbn-dummerstorf.de/en/

SCAN ME

ISEP 2025, Rostock-Warnemünde, Germany