WAGENINGEN
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THE 47TH ANIMAL NUTRITION RESEARCH FORUM

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PROGRAM BOOK

THE 47TH ANIMAL NUTRITION FORUM
~ANR 2025~

APRIL 25, 2025

WAGENINGEN CAMPUS

Impulse, Stippeneng 2, 6708 WE Wageningen, The Netherlands

THE 47TH ANIMAL NUTRITION RESEARCH FORUM

WELCOME

Dear colleagues,

With great pleasure and anticipation, we welcome you to the *47th Animal Nutrition Forum*, which will take place on Friday, April 25th, 2025, at Wageningen Campus (Impulse, Stippeneng 2, 6708 WE Wageningen, The Netherlands).
This scientific forum, originally initiated by **Wageningen University & Research, Utrecht University, KU Leuven, Antwerp University,** and **Ghent University**, aims to bring together young researchers in the field of animal nutrition. It provides a platform for them to share their knowledge, explore innovative ideas, and contribute to the advancement of this vital field.

We are proud to present an outstanding lineup of speakers who will share their insights and research findings, helping to advance our understanding of animal nutrition and its broader impact on animal welfare and health. Additionally, we are more than excited to introduce a special addition to this year's forum: **an interactive session**. This will be an engaging space where participants can actively contribute to the conversation through open discussions, questions, and answers. Aimed at fostering a dynamic dialogue, this session will focus on science communication in animal nutrition research, and we hope that this new addition will become a valuable tradition, allowing young researchers to share their perspectives and voice their opinions on important issues in the field.

We believe this event will be an invaluable opportunity for networking, learning, and sharing ideas with professionals from diverse institutions and universities. We encourage you to take full advantage of this forum and we are confident that you will leave not only with new knowledge and connections but also with more enthusiasm for your research and for the future of animal nutrition and health.

Looking forward to meeting you all in Wageningen!

With warm regards,
On behalf of Wageningen Livestock Research, the 47th ANR Forum Organizing Committee

Afroditi Lazarakou
Emily Teunissen
Gonzalo Vivares Martinez
Jordi Rijpert
Lisanne Koning
Roselinde Goselink
Teatske Nieuborg

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Organizing Committee

Afroditi Lazarakou

Emily Teunissen

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Jordi Rijpert

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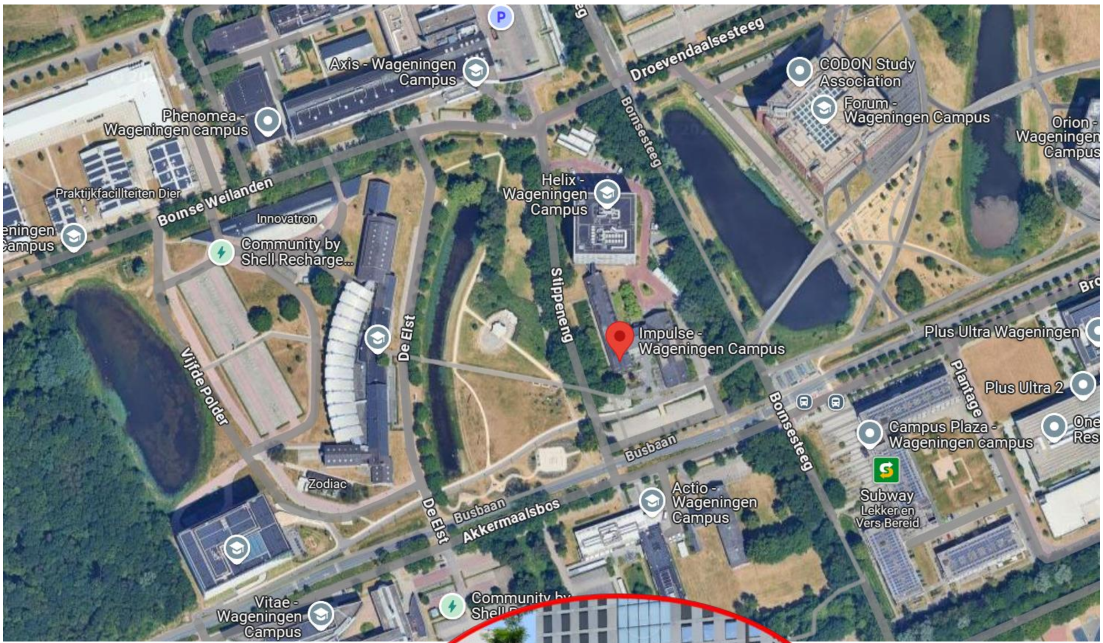
Teatske Nieuborg

Location-Map



Location 📍 **:**

Impulse, Building number 115.

Stippeneng 2, 6708 WE Wageningen

Parking 🅿 **:**

You can use P1, P2 or P3.

Preferred parking P2 is behind building 122 Zodiac. Just follow the parking signs.

General Schedule

Location	**Wageningen Campus**
	Impulse, Stippeneng 2, 6708 WE Wageningen, The Netherlands.
Time	**Activity**
09:00	Registration and coffee Poster Placement
09:30	Welcoming speech by Ernst van den Ende
09:45	Abstract presentation & poster pitches
10:45	Coffee break & poster session
11:30	Interactive session with Karel de Greef
12:30	Lunch
13:30	Keynote speaker Anja Janssen
14:00	Abstract presentations
14:30	Coffee break
15:00	Abstract Presentations
16:00	Closing speech
16:15	Closing reception with drinks and snacks

Detailed Schedule - Timetable

Location	Wageningen Campus	
	Impulse, Stippeneng 2, 6708 WE Wageningen, The Netherlands.	
Time	**Activity**	
09:00-09:30	Registration and Poster Placement Coffee	
09:30-09:45	Welcome speech	
	Managing director of Animal Sciences Group, Wageningen University and Research	dr.ir. Ernst van den Ende
09:45-10:45	**Session 1: Presentations & poster pitches**	
	O1: Gas and methane production from chicory, plantain, and birdsfoot trefoil at different regrowth stages: an in vitro study	Zhaoyang Cui
	O2: Impact of Low-Protein Feeding strategies and on Performance and Lysine Efficiency in Weaned Piglets	Yao Zhu
	O3: Potential of IgG from spray dried porcine plasma (SDPP) to bind bacterial pathogens associated with canine enteropathies.	Ilva Noa Stellingwerf
	Poster Pitches	P1-P6
10:45-11:30	**Coffee break and Poster session**	
11:30-12:30	**Interactive session**	
	To explore different perspectives on key issues in animal nutrition research in the form of propositions.	dr.ir. Karel de Greef dr. Roselinde Goselink
12:30-13:30	**Lunch break**	
13:30-14:30	**Session 2: Keynote speaker and Presentations**	
	In vitro techniques: how do food technologists measure protein digestion? ***Associate Professor, Food Process Engineering Group***	dr.ir. Anja Janssen

	O4: Effect of incorporating defatted black soldier fly meal on laying hen performance and egg quality.	Flore Blomme
	O5: The Role of Dietary Sulfur Amino Acids in Heat Stress Tolerance in Broilers	Herinda Pertiwi
14:30-15:00	**Coffee break**	
15:00-15:45	**Session 3: Presentations**	
	O6: Different feeding curves during gestation affect sows' body composition in the long term	Rafaella Carnevale
	O7: Milk urea, confounding factors and practical use to reduce dietary protein	Harmen van Laar
	O8: Effect of extruded linseed on methane emissions and rumen microbiota in two different diets	Joni Van Mullem
	O9: A population balance model to predict size reduction using a hammer mill of eight commonly used feedstuffs in animal feed formulation: practical application and sensitivity analyses	Jordi Rijpert
16:00-17:00	**Closing Ceremony**	
	Drinks & Snacks	

List of Oral Presentations

O1	Gas and methane production from chicory, plantain, and birdsfoot trefoil at different regrowth stages: an in vitro study	Zhaoyang Cui
O2	Impact of low-protein feeding strategies and on performance and lysine efficiency in weaned piglets	Yao Zhu
O3	Potential of IgG from spray dried porcine plasma (SDPP) to bind bacterial pathogens associated with canine enteropathies.	Ilva Noa Stellingwerf
O4	Effect of incorporating defatted black soldier fly meal on laying hen performance and egg quality.	Flore Blomme
O5	The role of dietary sulfur amino acids in heat stress tolerance in broilers	Herinda Pertiwi
O6	Different feeding curves during gestation affect sows' body composition in the long term	Rafaella Carnevale
07	Milk urea, confounding factors and practical use to reduce dietary protein	Harmen van Laar
O8	Effect of extruded linseed on methane emissions and rumen microbiota in two different diets	Joni Van Mullem
O9	A population balance model to predict size reduction using a hammer mill of eight commonly used feedstuffs in animal feed formulation: practical application and sensitivity analyses	Jordi Rijpert

List of Posters

P1	Impact of dietary soybean oil on gene co-expression networks in porcine muscle	Simara Fanalli
P2	Protein nutrition and damaging behavior in pigs	Jinda Glinubon
P3	Maternal amino acid supplementation during the dry period: effects on colostrum quality and calf development	Sarah Lievens
P4	Effect of chemical fractions of dairy cow's diet on mitigating efficacy of 3-nitrooxypropanol in vitro	Emiel Willaert
P5	Combining in vivo and in vitro evaluations to assess the potential of innovative emulsifier addition	Daphne Michels
P6	The effect of ribwort plantain (Plantago lanceolata) on enteric methane emission of dairy cattle during grazing	Lisanne Koning

Abstracts

O1

Gas and methane production from chicory, plantain, and birdsfoot trefoil at different regrowth stages: an *in vitro* study

Zhaoyang Cui[1], Jan Dijkstra[1], Gertjan Holshof[1,2], Wilbert Pellikaan[1]*

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Optimising ruminant nutrition is essential for reducing enteric methane (CH_4) emissions, which may be achieved by adopting unconventional forage species and harvesting at an earlier growth stage. This study investigated the *in vitro* gas production (GP), CH_4 production, and organic matter (OM) degradability of chicory (*Cichorium intybus*), plantain (*Plantago lanceolata*), and birdsfoot trefoil (*Lotus corniculatus*) across different regrowth weeks.
A 3 × 5 factorial design was implemented, comprising three forage species and five regrowth weeks (weeks 1 to 5, corresponding to 2 to 6 weeks after simultaneous regrowth from a single mowing date). Forage samples were incubated in triplicate with mixed rumen fluid from three lactating, rumen-cannulated Holstein-Friesian cows. The GP was assessed over 48 hours, CH_4 was measured at multiple time points, and OM degradability was determined after 48 hours. Data were analysed using a mixed-effects model, with species, regrowth week, and their interaction as fixed effects, and run as a random effect. Interactions between species and regrowth week significantly affected OM degradability as well as 48-h cumulative GP and CH_4 production (mL/g OM incubated). Chicory exhibited higher OM degradability than plantain and birdsfoot trefoil, with the difference narrowing from weeks 1 to 4 before widening in week 5. Chicory also demonstrated the highest GP (mL/g OM incubated), though interspecies differences diminished in weeks 4 and 5. Plantain had lower CH_4 production (mL/g OM incubated) than chicory in weeks 1 to 3 but did not differ significantly from birdsfoot trefoil. When expressed per unit of degraded OM, GP was highest in plantain, whereas CH_4 production was lowest in chicory. Chicory and plantain showed greater potential than birdsfoot trefoil in mitigating CH_4 emissions, with effects varying across regrowth weeks.

O2

Impact of Low-Protein Feeding strategies on Performance and Lysine Efficiency in Weaned Piglets

Yao Zhu[1], Chengcheng Li[1], Thomas Van De Putte[1], Sam Millet[2], Jeroen Degroote[1,]*

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[2] ILVO (Flanders Research Institute for Agriculture, Fisheries and Food), Animal Sciences Unit, Burg. Van Gansberghelaan 119, 9090 Merelbeke-Melle, Belgium.

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Feeding a low-protein (LP) diet to weaned pigs can improve gut health but may also impair growth performance. This study investigated the effects of protein level and feeding duration before transitioning to a common starter diet. A total of 101 weaned piglets (26-28 days, 7.02 ± 0.09 kg) were assigned to four dietary treatments: (1) HP10: high-protein (HP) diet (18.8% crude protein (CP), 12.4% standardized ileal digestible lysine (SID Lys)) for 10 days, followed by a common starter diet (17.9% CP, 11.8% SID Lys) until day 39; (2) HP18: HP for 18 days, then the starter diet; (3) LP10: low-protein (16.2% CP, 10.6% SID Lys) for 10 days, then the starter diet; (4) LP18: LP for 18 days, followed by the starter diet. Feed intake and body weight (BW) were monitored using electronic feeders and weighing stations. Lysine retention was calculated assuming a fixed lysine content per kg BW. And lysine efficiency was calculated as the ratio of lysine retention to SID Lys intake. LP18 showed a 17.7% reduction in average daily gain (ADG) compared to HP18 during days 0-18 ($P = 0.009$), with lower gain-to-feed in both LP groups than HP18 ($P = 0.017$). Both LP and HP10 had a higher lysine efficiency compared to HP18 during days 18-39 and 0-39 ($P = 0.003$ and $P = 0.002$, respectively). Additionally, lysine efficiency decreased as SID Lys intake per $BW^{0.75}$ increased during most periods (e.g., $y = 1.06 - 0.32x$, $P < 0.001$, $R^2 = 0.351$ for days 18-39), except days 0-10. In summary, prolonged LP feeding (18 days) reduced ADG and feed efficiency, whereas lower SID Lys intake improved lysine efficiency, except in early post-weaning when low ADG impaired efficiency.

O3

Potential of IgG from spray dried porcine plasma (SDPP) to bind bacterial pathogens associated with canine enteropathies.

Ilva Noa Stellingwerf [1,2], Coen Govers [2], Guido Bosch [1], Wouter Hendriks [1], Joost van Neerven [2]*

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Spray dried porcine plasma (SDPP) contains immunomodulating proteins, most importantly immunoglobulins. These have been shown to increase the diversity of the gut microbiome of e.g. fish, pigs and mice. Additionally, models of chronic enteropathies (CE) show addition of SDPP supports gut integrity, *e.g.* by its bactericidal effect on pathogenic bacteria, potential to increase microbial diversity in the gut, and the perceived increased production of anti-inflammatory cytokines in the intestines upon its addition to the diet.
To discover whether SDPP's effects derive from its interaction with host pathogens, we studied whether IgG from SDPP is able to bind and affect propagation of canine-specific bacterial and viral pathogens, all involved in the development of canine CE.
To discover SDPP's potential to target canine pathogens, the levels of IgG, IgM and IgA in two samples of SDPP were tested using a direct capture ELISA and found to be substantial, despite being heat sensitive: approx. 25%, 9% and 3% of total protein content. With a further focus on IgG, binding to 4 bacterial species, 2 strains of each species, associated with canine CE (*C. perfringens, E. coli, E. faecalis* & *S. canis*) was tested. Further focus was put on viral pathogens associated with CE, specifically canine parvovirus (CPV), associated with both acute and chronic gut damage. ELISAs showed immunoglobulins from SDPP bound CPV. In addition to binding, a neutralization assay was set up. Here, SDPP was pre-incubated with CPV, then added to cells susceptible to CPV infection. The control received CPV only. This assay showed significant neutralization of canine parvovirus by SDPP, for 10, 100 and 400 μg/mL SDPP (unpaired t-test comparing concentration to control; $P \geq 0.05$).
To conclude, immunoglobulins in SDPP were shown to bind both bacterial and viral pathogens associated with canine enteropathies, thus forming a potentially beneficial addition to the diet of dogs suffering from CE.

O4

Effect of incorporating defatted black soldier fly meal on laying hen performance and egg quality

Flore Blomme[1], Evelyne Delezie[1], Katrien Van Biesen[2]*

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Due to the high protein content and low environmental footprint, black soldier fly meal can be considered a good alternative protein source.The aim was to investigate the effect of partially replacing soybean meal with defatted black soldier fly meal (DBSFM).
240 Lohman Brown hens of 76 weeks were housed in enriched cages with each 5 laying hens. The trial consisted of 4 dietary treatments, each replicated 12 times. The trial lasted 16 weeks. The diets were wheat-soybean meal based. Soybean meal was partially replaced by DBSFM, with T1 as the control diet and increasing replacement levels of 20% (T2), 35% (T3) and 50% (T4).
Feed intake, laying percentage and mean body weight of the laying hens were determined. Egg mass and feed conversion ratio (FC) were calculated every period. Each 21st day of a period and at the start and end of the trial, 30 eggs of every dietary treatment were collected to determine external- and internal egg quality. Feacal droppings of laying hens fed with T1 and T4 were analyzed for dry matter. At the end of the trial eggs of T1 and T4 were analyzed on fatty acid composition (FA). A taste panel was organized to test omelets on organoleptic characteristics.
Fatty acid composition of the eggs, faecal dry matter and performances were not significantly affected, with the exception of the FC of T3 which was significantly higher compared to T1 ($P = 0.03$). Since performance of T4 was not affected, no explanation can be given for this result. Incorporating DBSFM had no significant effect on organoleptic characteristics of the egg and egg quality, with the exception of yolk colour, where yolk colour was significantly higher in T3 compared to T2 and T4 during period 1 ($P<0.001$). Therefore, DBSFM can replace soybean meal up to 50% in layer feeds without compromising performance, egg quality or taste of the eggs.

O5

The Role of Dietary Sulfur Amino Acids in Heat Stress Tolerance in Broilers

Herinda Pertiwi[1], Huaiyong Zhang[1], Stefaan De Smet[1], Jeroen Degroote[1]*

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Heat stress (HS) poses a significant challenge to broiler production, leading to impaired growth performance, feed efficiency, animal health, and behaviour. Sulfur amino acids (SAA), primarily methionine and cysteine, play crucial roles in growth, metabolic regulation, and antioxidant defence, suggesting their potential in mitigating HS-induced performance losses. This study extensively reviewed the impact of dietary SAA supplementation under thermoneutral (TN) and chronic HS conditions, providing a quantitative assessment of performance deviations and evaluating the effectiveness of SAA inclusion in alleviating HS-induced stress. A comprehensive literature search was conducted across PubMed, Scopus, and Web of Science using relevant keywords related to poultry, nutrition, and HS. A total of 131 peer-reviewed studies (1988–2024) were selected based on predefined inclusion criteria, resulting in 159 experiments categorized as cyclic (n = 111) or constant (n = 48) chronic HS. Experimental data included average daily gain (ADG), average daily feed intake (ADFI), and feed conversion ratio (FCR) under TN and HS conditions. Performance deviations were calculated as percentage differences between HS and TN groups at equivalent dietary SAA levels (0.58%–1.17% standardized ileal digestible methionine plus cysteine). Linear regression analysis revealed that increasing dietary SAA significantly mitigated HS-induced performance losses in constant HS conditions, with higher SAA levels correlating with reduced declines in ADG (coefficient = -0.60, P = 0.028), ADFI (coefficient = -0.39, P = 0.037), and improved FCR (coefficient = 0.37, P = 0.050). In contrast, cyclic HS conditions didn't demonstrate any significant effects, indicating that birds under cyclic HS may exhibit acclimatization, reducing the impact of SAA supplementation. The study highlights the potential of dietary SAA in mitigating the negative effects of prolonged HS while emphasizing the need for further research to refine dietary recommendations for broilers under heat stress conditions.

O6

Different feeding curves during gestation affect sows' body composition in the long term

Rafaella Carnevale[1,2,3], Nathalie Nollet[4], Cesar Garbossa[2], Geert Janssens[3], Sam Millet[1,3], An Cools[1,4]*

[1] Department of Veterinary and Biosciences, Ghent University, Belgium

[2] Department of Animal Nutrition and Production, University of São Paulo, Brazil

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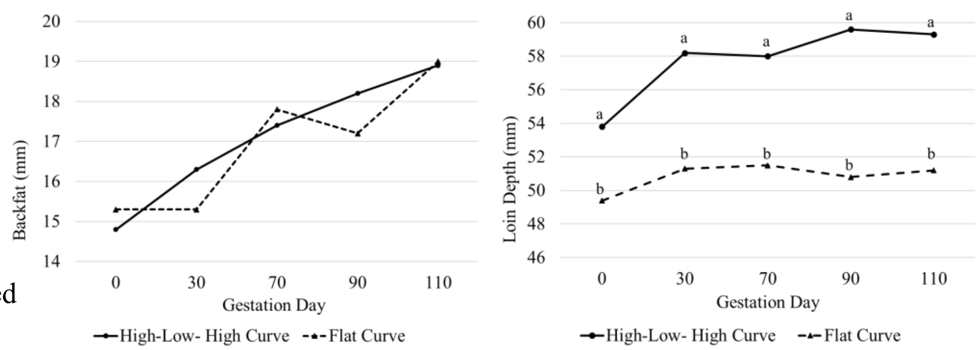
Overfeeding sows leads to peripartal issues and reduced lactation feed intake, while underfeeding may cause muscle and fat breakdown. Both over- and underfeeding negatively impact sows' longevity. Farmers try to cope with the sow's nutritional needs by either feeding a constant amount of feed (flat) or feeding a higher amount in early/late gestation, and a lower amount in mid gestation (high-low-high, HLH). We designed a cross-sectional study to check correlations between feeding strategies and sow's body condition. In total, 1371 sows from 36 farms were enrolled in a cross-sectional study, with 244 sows from 11 farms being fed a flat curve (same amount of daily feed allowance throughout gestation) and 1073 sows from 25 farms being fed a HLH (reduction in daily feed allowance between days 35-70 of gestation). At one time point, sows of different batches were measured. The batches were in different stage of gestation: 0, 30, 70, 90, and 110 days of gestation. Backfat (BF) and loin depth (LD) were measured with an ultrasound mode B at P2 position. Data was analyzed by ANOVA. The average LD was higher in sows fed HLH ($P<0.05$) than sows fed a flat curve, regardless period of gestation. The BF was similar for both groups at all time points as well as the average BF regardless of the period of gestation (figure 1). Farms using HLH had sows with greater loin depth, though it's unclear if this stems from the feeding scheme or other confounding factors. Further research will need to clarify how LD interacts with sows' metabolism at different moments of gestation and what is the ideal range of LD and BF to optimize maternal health and reproductive performance.

Figure 1. Backfat and loin depth of sows at different gestational ages feed a high-low-high or a flat feed curve.



O7

Milk urea, confounding factors and practical use to reduce dietary protein

Harmen van Laar, [1] *ChatGPT (for text condensation)*

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The Cow and Protein Project (Koe & Eiwit) helps dairy farms lower dietary protein levels to 155 g/kg DM. Milk urea levels are indicative of the protein supply status of lactating cows and are used to guide nutritional decisions. To understand the reliability of milk urea as an indicator a qualitative literature review explored the relationship between protein nutrition and milk urea and potential confounding factors.

Urea, a nitrogen-rich molecule (46.7% nitrogen), is a key excretion pathway for surplus nitrogen. It forms in the liver from dietary nitrogen fermented in the rumen or amino acid breakdown and circulates in the blood. Milk urea reflects dietary protein, with approximately a 4 mg/dL increase per 10 g/kg DM rise in crude protein in the diet. However, this relationship is affected by several factors:

- **Timing of feeding:** Milk urea mirrors blood urea, which fluctuates with feeding patterns, especially protein-rich meals.
- **Salt levels:** Higher salt intake increases water consumption, reducing blood and milk urea. Adding 500 g salt can lower milk urea by ~4 mg/dL. Grass and maize silage diets also differ in salt content.
- **Genetics:** Milk urea levels vary between cows. Breeding values for bulls range from +6 to -6 mg/dL, influencing offspring levels. Farms in the top 10% vs. bottom 10% for breeding values differ by ~1 mg/dL.
- **Heat stress:** Milk urea gradually rises with a temperature-humidity index up to 74 and steeply increases beyond.
- **Protein type:** Rumen degradable protein impacts milk urea more than rumen undegradable protein. A 100 g increase in rumen protein balance (OEB) raises milk urea by ~2 mg/dL.
- **Young stock and dry cows:** Milk urea reflects lactating cow diets only, whereas the entire herd's protein level must be considered.

In conclusion, milk urea is a valuable indicator of protein nutrition but requires careful interpretation considering these factors.

O8

Effect of extruded linseed on methane emissions and rumen microbiota in two different diets

Joni Van Mullem[1,2,], J. Jeyanathan[2], Nico Peiren[1], Veerle Fievez[2], Leen Vandaele[1]*

Extruded linseed (EL), rich in polyunsaturated fatty acids, has already demonstrated significant potential for enteric methane mitigation. This study aimed to assess the impact of EL on production parameters, methane emissions and ruminal microbiota when supplemented into two different diets. A 2X2 cross-over experiment was conducted using 39 lactating Holstein dairy cows (155 ± 50 days in milk, 32.1 ± 5.4 kg milk/day). Four dietary treatments were included: 1) maize silage (MS)-based diet (MS:grass silage (GS) of 75:25 on dry matter base), 2) MS diet supplemented with EL (400g crude fat/day), 3) GS-based diet (MS:GS of 25:75 on dry matter base), and 4) GS diet supplemented with EL (400g crude fat/day). Data on dry matter intake (DMI), milk production, milk composition and methane emissions were collected. Rumen fluid was sampled for metataxonomic analysis targeting four domains: bacteria, archaea, protozoa, and fungi. Supplementation of EL reduced DMI (MS diet: 24.2 to 23.7 kg; GS diet: 23.9 to 23.0 kg, $P < 0.05$) but increased milk yield and fat-and-protein corrected milk yield (MS: 1.0 kg and 0.9kg; GS: 1.2 kg and 1.5 kg; $P < 0.01$). Milk protein and urea nitrogen ($P < 0.05$) were reduced by EL, while increasing lactose ($P < 0.01$) in both diets, with milk fat decreasing only in the GS diet ($P = 0.064$). Methane production (g/day) and intensity (g/kg milk, g/kg fat-and-protein corrected milk) decreased ($P < 0.01$) by 5% and 7% across both diets because of the addition of EL. Alpha and beta diversity was either affected by diet (Bacteria and Fungi), by diet and treatment (Archaea) or not significantly affected by diet nor treatment (Protozoa). To conclude, EL supplementation reduced methane production and intensity, with preliminary microbiota results suggesting a diet-dependent interaction.

O9

A population balance model to predict size reduction using a hammer mill of eight commonly used feedstuffs in animal feed formulation: practical application and sensitivity analyses

Rijpert J.H.M.[1,2], Bosch G. [1], Illera-Rodriguez M. [3], Bastiaansen T.M.M.[1,2], Gerrits W.J.J. [1]*

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Size reduction of feedstuffs is a key first step in the production of animal feed. We constructed a model to predict particle size reduction using a population balance approach for the individual milling of eight commonly used feedstuffs in a hammer mill. Driving variables for prediction of the outflowing particle size distribution were chosen to be the inlet mass feed rate, product density, hammer rotational speed, mill residence time and sieve aperture size. The model was calibrated to data of corn, barley, faba beans, soybean meal, rapeseed meal, sunflower meal, wheat middling's and corn DDGS ground using different machine intensities. The resulting change in particle size distribution was modelled using a set of equations describing breakage kernel, breakage rate and screen selection functions, where eight model parameters were used to ensure physical relevance. Global sensitivity analyses of the model indicated that mill residence time explained the majority of model output variance ($R^2_{adjusted}$ of 0.6 to 0.8, depending on particle size). Of the model parameters, those describing particle breakage gave the highest reduction in predicted root mean square error in explaining particle size reduction. Maximum likelihood estimation of the model parameters was performed to identify a general set of parameters that describes the milling behaviour of the evaluated feedstuffs. Model adherence to validation data was generally strong, where increasing grinding intensity by varying driving variables lead to smaller resulting particle sizes. The presented model can be used as a practical tool to predict changes to particle size when milling individual feedstuffs in a hammer mill. Moreover, analysis of the model has indicated next steps for improving predictions, especially for smaller particle sizes.

P1

Impact of dietary soybean oil on gene co-expression networks in porcine muscle

Simara Fanalli[1,2], Richard Crooijmans[2], Aline Cesar[1]*

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Dietary fat influences gene expression and metabolic changes in livestock, depending on both the type and amount of fat consumed. Intramuscular fat (IMF) deposition is a key trait affecting meat quality and metabolic processes in pigs. The objective of this study was to deepen our understanding of the relationship between different diets of soybean oil levels (1.5% SOY1.5 or 3% SOY3.0), gene expression, and the composition of deposited fatty acids in IMF. The study (protocol 2018.5.1787.11.6, CEUA 2018-28-Brazil) used 33 skeletal muscle samples from immunocastrated Large White pigs. RNA was extracted and sequenced (Illumina), with quality control (FastQC v.0.11.8) and preprocessing (Trim Galore v.0.6.5). Alignment and quantification were performed using Bowtie2 (v.2.4.3) and RSEM (v.1.3.1). Weighted gene co-expression network analysis (WGCNA) was applied using TPM and FA deposition data adjusted by a linear model. The modules with the highest correlation for each diet were further analyzed using ClueGo (v.2.5.10) in Cytoscape (v.3.10.2). The module with the highest correlation for each diet was analysed using ClueGo v.2.5.10, a Cytoscape v.3.10.2 plugin. In the SOY1.5 group, we identified the Cyan module ($r = -0.63$ with Atherogenic Index), while in the SOY3.0 group, we found two modules: Darkred ($r = -0.68$) and Black ($r = +0.68$) correlated with palmitoleic acid. The *Cyan* module in SOY1.5 was associated with biological processes and pathways involving the genes *PLA2G2D* and *PLB1*, linked to linoleic acid metabolism; alpha-linolenic acid metabolism. In contrast, in SOY3.0, we found a positive correlation between palmitoleic acid and pathways related to glucose and lipid metabolism, insulin and adipocytokine signalling, and insulin resistance. Conversely, palmitoleic acid showed a negative correlation with protein-lysine methylation and lysine degradation pathways. These findings confirm that diet affects not only fatty acid deposition but also molecular regulation.

P2

Protein nutrition and damaging behaviour in pigs

Jinda Glinubon[1,2,3], J. Elizabeth Bolhuis[2], Alfons J. M. Jansman[3], Walter J. J. Gerrits[1]*

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This project review explores the effects of amino acid (AA) supplementation and dietary fibre (DF) on damaging behaviour in pigs, particularly tail biting, with a focus on their roles in serotonin production, gut microbiota interactions, and behavioural regulation. Tryptophan (Trp) serves as a precursor of serotonin, a key neurotransmitter involved in mood, aggression, stress, and feed intake. To enter the brain, Trp competes with large neutral amino acids (LNAA) for the same transporter across the blood-brain barrier. Research suggests that increasing the Trp:LNAA ratio enhances serotonin production, potentially reducing aggression and stress-related behaviours. While most Trp is absorbed in the intestine, some reaches the colon, where it is metabolized by the gut microbiota. Interestingly, the gut microbiota plays a crucial role in the bidirectional interactions between the gut and the nervous system, known as the microbiota-gut-brain axis, which influences mood and behaviour. This complex relationship is further supported by DF, which promotes gut microbiota diversity and affects nutrient absorption. The bulk properties of DF influence gastrointestinal transit time and increase viscosity, leading to prolonged retention in the gut and improved nutrient digestibility by enhancing exposure to digestive secretions and contact with epithelial cells in the intestine. Furthermore, DF is partially fermented by gut microbiota in the large intestine, producing short-chain fatty acids that serve as energy sources and promote satiety. It has been hypothesized that certain fibres may help reduce harmful behaviours. Therefore, this project review aims to examine the effects of Trp supplementation on damaging behaviour, its interaction with other LNAA, and the role of dietary fibre in shaping behaviour. Understanding these factors could support the development of nutritional strategies to improve animal welfare and reduce behavioural disorders in pig production.

P3

Maternal amino acid supplementation during the dry period: effects on colostrum quality and calf development

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Colostrum is the first milk a cow produces after calving and is crucial for neonatal immunity due to its high concentration of immunoglobulin G (IgG). Maternal nutrition during the dry period can influence both colostrum composition and passive immunity transfer. In particular, supplementation with amino acids such as methionine and lysine may enhance colostrum quality and improve calf performance. This trial investigates the effect of amino acid supplementation during late gestation on colostrum quality and calf development. Sixty Holstein-Friesian cows were randomly assigned to a control group (n = 30) or a treatment group (n = 30) receiving rumen-protected methionine and lysine from 28 days prepartum. At birth, all calves were fed colostrum according to a standardized protocol and allocated to one of four groups (n = 15 each), based on maternal diet and colostrum source: (1) control/control, (2) control/treated, (3) treated/control, and (4) treated/treated. This 2x2 design allows the independent assessment of maternal supplementation and colostrum composition on neonatal outcomes. Cows were monitored for feed intake, dry period length, calving ease, colostrum yield and quality (including immunoglobulins, nutrient profile, and microbial composition). Blood and fecal samples were collected to assess metabolic and microbiome changes. Calves were followed up to four months for passive immunity transfer, growth, feed intake, and health status. The trial began in February 2025; results are expected by the end of 2025. We expect that amino acid supplementation will increase colostrum quality, particularly IgG concentration, and positively influence calf development, with effects depending on both the maternal treatment and the colostrum source.

P4

Effect of chemical fractions of dairy cow's diet on mitigating efficacy of 3-nitrooxypropanol in vitro

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Enteric methane emissions from cattle contribute to global warming. The feed additive 3-nitrooxypropanol (3-NOP) has already demonstrated a significant methane reduction potential. However, the mitigating effect of 3-NOP may be influenced by the chemical fractions of the basal diet. This study aims to investigate if the known interaction from in vivo research can also be observed in vitro to validate the in vitro set-up and make the exploratory in vitro screening scientifically more robust. In the first in vitro batch experiment, the effect of 3-NOP on relative CH_4 production (methane/total VFA, mol/mol) was evaluated at a dose of 0.760 mg/gDM, supplemented in 25 substrate combinations (5 forage and 5 concentrate mixtures, to broaden the range of the chemical fractions) under standardised in vitro conditions. All diet combinations had a forage-to-concentrate ratio of 65:35. The 5 forage mixtures consisted of pressed beet pulp (10%) and a varying grass silage to maize silage ratio (90%). The experimental design resulted in highly correlated covariates, so the linear mixed models only included an overall mean and a single chemical variable. Individually, NDF and starch content of the forage and crude ash content affected the mitigating effect of 3-NOP, expressed as the relative mean difference (%) to the control. A 1% (10 mg/gDM) increase in substrate NDF_{forage} and crude ash content from their mean, decreased the efficacy of 3-NOP by 8.62%-units ($P<0.05$) and 2.73%-units ($P<0.05$), respectively. A 1% (10 mg/gDM) increase in substrate $starch_{forage}$ content from its mean, increased the efficacy of 3-NOP by 0.742%-units ($P<0.05$). So, the efficacy of 3-NOP is also influenced in vitro by the dietary chemical composition as observed in vivo. Further analysis will be conducted to quantitatively compare the variation in the efficacy of 3-NOP observed in vitro with the variation and interactions associated with the basal diet in vivo.

P5

Combining *in vivo* and *in vitro* evaluations to assess the potential of innovative emulsifier addition

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Poultry meat consumption rises yearly, attributed to chicken's nutritional composition, including essential amino acids, and lower lipid content. However, its production has a substantial environmental impact needing reduction to meet consumer demands sustainably. One possible solution to this problem is to improve macronutrient digestion in broilers. Broiler trials aim to find cost-effective and sustainable meat production methods. According to the three R principle, *in vitro* evaluations should precede *in vivo* testing. Often, feed technology researchers do not perform extensive *in vitro* evaluations due to a lack of protocols accurately describing the growing broiler's gastrointestinal tract. Therefore, our research aimed to evaluate nutrient digestibility of differently designed diets by complementing both *in vitro* and *in vivo* evaluations. The effect of upfront emulsification *(i)* versus separate oil and emulsifier addition *(ii)* was researched. Broiler diets were supplemented with oil and an emulsifier mix (LEX) separately *(i)* or with an o/w emulsion *(ii)*. Body weight and lipid and protein digestibility were measured *in vivo*. Identical diets underwent *in vitro* digestion using adapted parameters to simulate the growing broiler GIT, providing mechanistic insights into protein, lipid, and starch digestion kinetics. *In vitro* lipolysis kinetics experiments showed a sigmoidal behaviour with shorter lag times in diets containing more LEX and/or diets *(ii)* compared to diets *(i)*. Additionally, different *in vitro* lipolysis and proteolysis kinetics in diets lacking LEX (control) indicated interactions between lipids and proteins. These *in vitro* observations could be linked to significantly ($P<0.05$) better performances of broilers fed o/w-emulsion-supplemented diets *(ii)*. Although, i*n vivo* trials did not show significantly higher lipid or protein digestibilities for O/W diets. This study demonstrated that *in vitro* digestion evaluations, using appropriate conditions to simulate the broiler gastrointestinal tract, can reduce the need for *in vivo* testing and complement *in vivo* data by providing insights into digestion kinetics.

P6

The effect of ribwort plantain (*Plantago lanceolata*) on enteric methane emission of dairy cattle during grazing

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Herb-rich grasslands are of increasing interest due to potential properties of reducing enteric methane (CH_4) emission and increasing biodiversity. The objective of this study was to compare enteric CH_4 emission of cows grazing on perennial ryegrass (*Lolium perenne*, LP) pastures with and without ribwort plantain (*Plantago lanceolata*, PL). Thirty-two dairy cows were blocked and assigned to one of the treatments: LP and LP with PL (LP-PL) in Duplo on adjacent pastures which received the same grassland management. Cows were continuously grazing for four weeks per period; two weeks of adaptation and two weeks of measurement period. The trial was repeated three times in 2022: May-Jun (period 1), Jul-Aug (period 2) and Sept-Oct (period 3). Enteric CH_4 emission was measured using GreenFeed systems (C-lock Inc.). Fresh grass intake was estimated using the net energy requirement (VEM) calculations. A restricted maximum likelihood (REML) analysis was done with pasture (1 / 2) and botanical composition (LP / LP-PL) as fixed effects and block as random effect. The CH_4 production and yield was significantly higher for LP-PL compared to LP ($P < 0.001$ and $P = 0.046$, respectively, Figure 1). This study did not show a CH_4 reduction potential of PL, but that may be due to the low proportion of PL in the pasture and the possibility for cows to select while grazing.

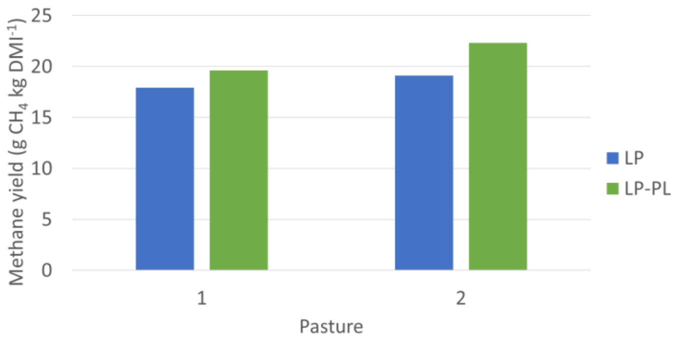

Figure 1. Methane yield (g CH_4 kg DMI^{-1}) per treatment group; Pasture 1 or 2 and with PL (LP-PL, in green) or without PL (LP, in blue).

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