FACULTY OF
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48th ANR Forum

Merelbeke, 17 April 2026

GHENT
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Table of Contents

WELCOME

It is an understatement that things have changed since the first edition of this forum in 1976. Science has become increasingly international. Topics have shifted from mainly focused on technical performance of production animals to a One Health dialogue, where animal nutrition can no longer be separated from concerns on climate change, biodiversity and human and animal health. The reason why this yearly event exists for 50 years but only has its 48th edition, is due to the corona pandemic. Respected initiatives such as the UN Environment Programme have listed intensified agriculture and livestock production, biodiversity loss, pollution and climate change as drivers of pandemic outbreaks.

There is, therefore, an urge to find integrated solutions for the sustainable care of animals, whether they are kept for production, company, leisure or ex-situ conservation and education. Animal nutrition research offers a wealth of opportunities to accomplish that. Let us make the ANR Forum the event to have an unbiased-as-possible discussion, learning from the past, living in the present, thinking about the future.

A warm welcome to this forum with hopefully fruitful discussions.

The organising committee,

Geert Janssens – Ruben Van Gansbeke – Donna Vanhauteghem

SNAK – Studies in Nutrition across the Animal Kingdom

Department of Veterinary and Biosciences, Faculty of Veterinary Medicine, Ghent University.

PROGRAM

9:30		**WELCOME & REGISTRATION**
10:00	Janssens et al.	Half a century of commuting to share nutrition science
10:15	Mertens et al.	Development and on-farm implementation of a multidimensional sustainability assessment and benchmarking tool for pig and broiler production
10:30	Zom et al.	The effect of dairy farm extensification under agri-environmental management on nitrogen excretion and farm revenues: A modelling approach
10:45	van Laar et al.	Impact of VEM2022 in the farm specific excretion model BEX for NL
11:00	Zhang et al.	Enhancing the fiber degradation efficiency in dairy cattle rumen through engineered bacterial communities
11:15	Liang et al.	Nutritional evaluation a forage quality gradient: Validation of Forage Quality Score (FQS) through rumen dynamics, nutrient digestibility, and energy retention in beef cattle
11:30	Su et al.	Influence of inoculum type and substrate on the efficacy of 3-nitrooxypropanol in inhibiting methane production in vitro
11:45	Willaert et al.	Efficacy of 3-NOP in mitigating CH4 production in relation to the chemical composition of the in vitro substrate
12:00		**LUNCH**
13:15	Teunissen et al.	Dietary characteristics affect enteric methane and odour formation in pigs: insights from colon digesta incubation and analysis of exhaled air
13:30	Zhu et al.	The effect of choice-feeding between high- versus low- crude protein diet on feeding behaviour in weaned piglet
13:45	Puthiyottil et al.	Conditioning feed influences starch digestion kinetics in an ingredient dependent manner
14:00	Kurpan et al.	Zootechnical and physiological impacts of adding a concentrated solution of Chlorella vulgaris FAM28093 to feed for post-weaning piglets: a pilot study
14:15	Gudeta et al.	Effect of the protein content of waste substrates on the production and composition of earthworms as a feed protein source
14:30	Kraft et al.	Black soldier fly larvae-derived chitin and chitosan as a strategy against enterotoxigenic Escherichia coli-infections in piglets: an in vitro study
14:45	Buyse et al.	The potential of bile acids in avian metabolism
15:00		**BREAK**
15:30	Glinubon et al.	Interactive effects of dietary tryptophan and large neutral amino acids on growth performance and behaviours in weaned piglets
15:45	De Oliveira et al.	Dietary fiber inclusion in the diet influences behavior and promotes satiety in group-housed gestating sows
16:00	Kleinherenbrink & Bosch	The satiating potential of mice versus dry food and moist food in cats
16:15	Zhang et al.	In vitro mineral accessibility in commercial vegetarian and vegan extruded dog foods
16:30	Sun et al.	The effect of additional calcium and insoluble fibre on performance and calcium metabolism in weaned piglets
16:45	Kalmar et al.	Species-specific effects of dietary calcium on psittacine blood biochemistry
17:00	Janssens	Wrap-up & discussion about the future of the ANR Forum
17:00		**RECEPTION**

SESSION 1

Half a century of commuting to share nutrition science

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Introduction: In 1976, nutrition scientists from The Netherlands and Belgium agreed to initiate a yearly gathering of academics working in the field of nutrition science. The aim was to create a forum for especially young – a term still to be defined – scientists to share their (partially) completed studies, and expose them for collegial debate. Fifty years later, the founders may have been astonished that their creation is still alive, albeit with another name. The round number of 50 years fed the urge for a retrospective on a "moving" history.

Material and Methods: In a building that used to be called Zootechnical Institute, at the back of closets in a room that used to be the office of the late professor Roland De Wilde, a pile of booklets and letters was identified as a carefully stored collection of abstract books of the Animal Nutrition Research Forum aka *Studiedag der Nederlandse Voedingsonderzoekers*. The pile was extracted and dissected into separate editions, and supplemented with 21st century editions (either cellulose or bytes) that were haphazardly stored by the first author. The latter kindly asked the coauthors to distil the core information such as publication year and species from each abstract and to precipitate it in Microsoft Excel. Presenters' character traits were not considered in the analysis due to risk of complexation. Spearman correlation analysis in SPSS was used to identify associations between year, country and species. Mentioned correlations are significant at $P < 0.05$, unless specifically mentioned they are not significant (NS).

Results and Discussion: Progessing age of the forum was positively correlated (r = 0.748) with the number of Belgian contributions versus no significant correlation with Dutch contributions (r = -0.111; NS). The authors dare not draw conclusions though. Over the years, the number of contributions by a country (Belgium or The Netherlands) was significantly associated with species: Belgian participation is typically associated with abstracts on companion animal (r = 0.674) and "others" (r = 0.513), whereas The Netherlands' participation is rather associated with ruminants (r = 0.605) such dairy cattle and sheep. However, the number of abstracts on sheep significantly declined over the years (r = -0.564), maybe due to the declining use of sheep as a model species in ruminant feed evaluation. The same occurred for rats (r = -0.427), potentially reflecting less focus on fundamental research or models for human nutrition. By contrast, there were significant increases in the number of abstracts on companion animals (r = 0.630) and monogastric livestock (r = 0.376; mostly caused by poultry) over the years. Topics also shifted through time, with studies on performance (only) making place for studies involving environmental impact, gut health and food safety (e.g. mycotoxins), maybe to be summarised as attention to One Health aspects.

The number of abstracts in function of year could be fitted with a 2nd order polynomial function, with an increase in abstracts until a peak production around 2005, followed by a slow decline due to yet to be uncovered factors.

References: Abstract books of (almost) all years of however this meeting has been called over the years.

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

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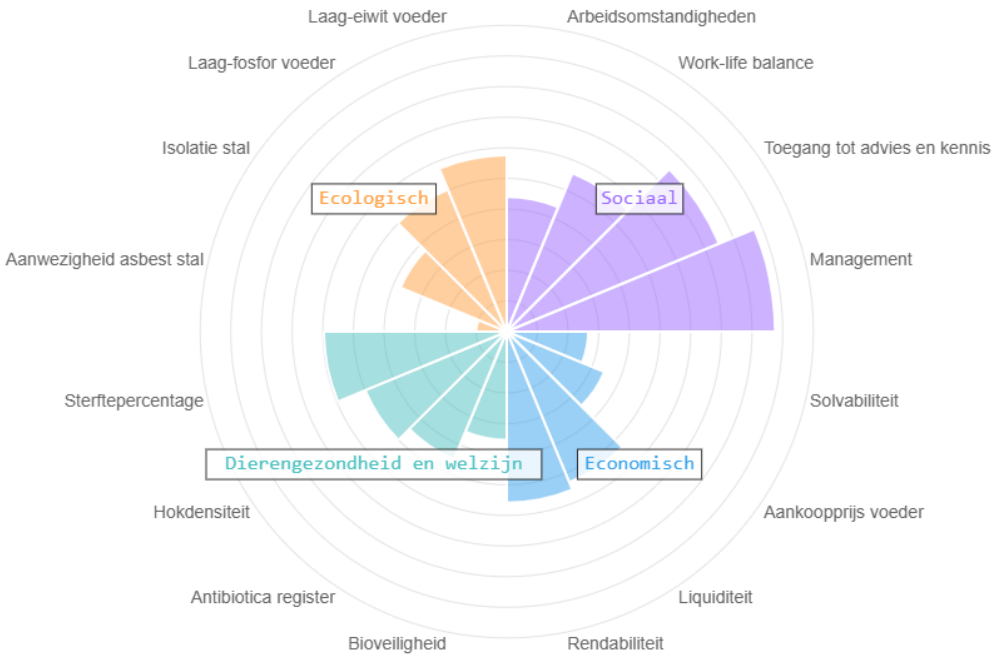
Introduction: Sustainable animal production is a multidimensional concept encompassing animal welfare, environmental impact, economic viability and social well-being. Despite increasing societal and policy attention, livestock farmers often lack clear insight into their overall sustainability performance. Most existing frameworks focus on a single sustainability aspect – typically economic and ecological impact – leaving animal welfare and social dimensions underrepresented. Given that improvements in one dimension can entail trade-offs in others, a holistic perspective is essential for informed decision-making.

Material and Methods: To address this gap, we aimed to develop a multidimensional sustainability assessment, information and benchmarking tool for pig and broiler production farms. The tool provides structured insight into farm performance across the four key sustainability pillars: animal health and welfare, environment, economy and social well-being. The online survey tool with automatic and personalized reporting was developed and evaluated in close collaboration with farmers, experts and stakeholders. Within each sustainability dimension, a set of carefully selected key performance indicators (KPIs) were defined, based on existing questionnaires and tools (e.g. AMCRA, Biocheck, Ruckli et al., 2022) as well as expert discussions. This resulted in 9 KPIs for animal health and welfare, 8 for environment, 9 for economy and 8 for social well-being. Several indicators across the sustainability dimensions are linked to feed use and composition, such as feed conversion ratio, feed cost, and the protein and phosphorus content of the feed. The questionnaire entails 56 questions and takes between 30 to 90'. The report consists of 3 parts: 1) global overview figure of the two best and least scoring KPIs per sustainability pillar, 2) summary figure for each sustainability dimension presenting the scores of all KPIs, categorized into strengths and areas for improvement, for which recommendations are provided, and 3) more detailed information for all KPIs, including benchmarking across all participating farms. Rather than providing a weighted average that might mask underlying issues, the tool focuses on identifying specific strengths and critical points of attention for each farm. By evaluating the KPIs against predetermined reference values, relative scores are calculated. This results in a personalized report with targeted recommendations corresponding with those indicators requiring attention. Additionally, the tool fulfills a significant industry demand for benchmarking, allowing farmers to compare their results with peer groups, thereby providing an extra incentive for adoption. The applicability and suitability of the tool were evaluated through implementation on 33 pig farms and 23 broiler farms in Flanders.

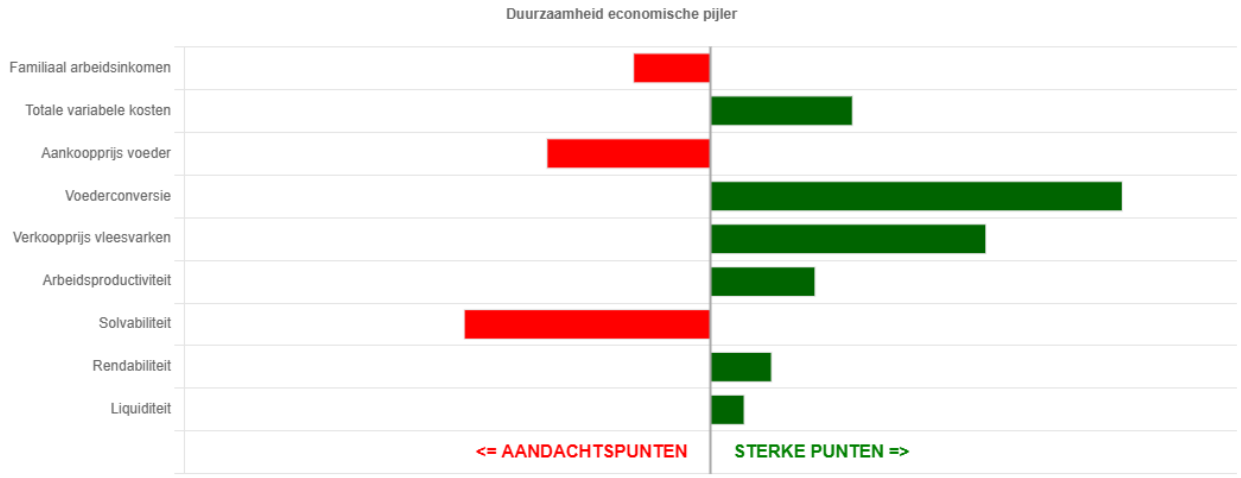
Results and Discussion: In general, pig farms obtained high scores for the KPIs feed conversion ratio, mortality rate and barn insulation, whereas lower scores were observed for sustainable water use.

Considerable variability was observed for most economic KPIs (e.g. family labor income and labor productivity) and for total energy use. Broiler farms generally scored high on liquidity, biosecurity and dead-on-arrival, while lower scores were observed for profitability and sustainable water use. Similar to pig farms, high variability was found for most economic KPIs (e.g. feed cost) and for renewable energy use. For the social dimension, both pig and broiler farmers generally scored well, with the highest scores achieved for management and access to advice and knowledge, whereas average scores were lower for networking and work-life balance.

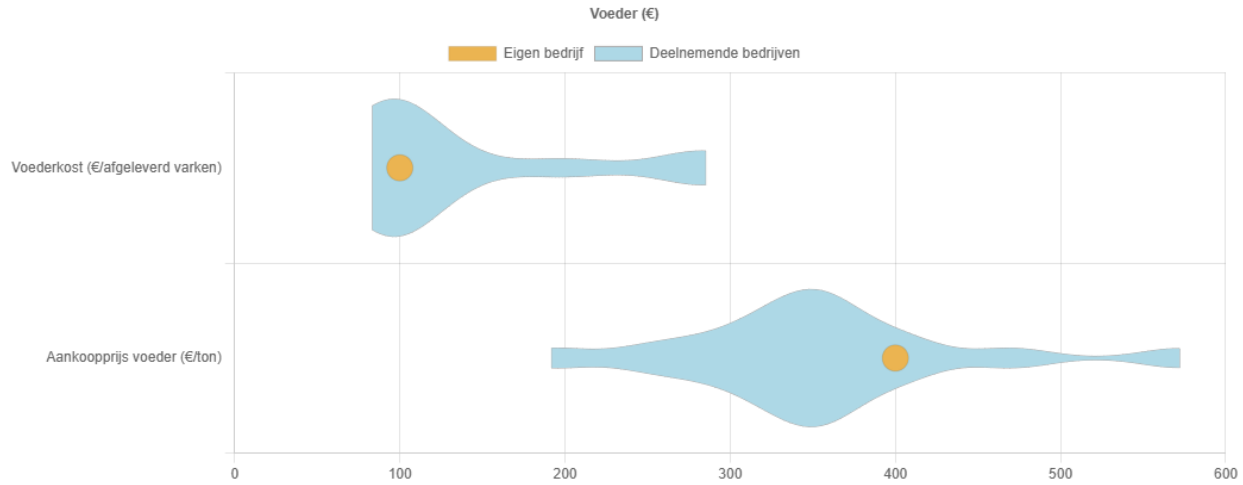
The results demonstrate the feasibility of a multidimensional, non-aggregated sustainability assessment approach and highlight the variability in performance both within and across sustainability pillars.



A global overview figure shows the two best and least scoring KPIs per sustainability pillar for a specific farm.



A summary figure for each sustainability dimension presents the scores of all KPIs, categorized into strengths and areas for improvement.



More detailed information, including benchmarking across all participating farms, is provided for most KPIs.

References: Ruckli, A. K., Hörtenhuber, S. J., Ferrari, P., Guy, J., Helmerichs, J., Hoste, R., Hubbard, C., Kasperczyk, N., Leeb, C., Malak-Rawlikowska, A., Valros, A., & Dippel, S. (2022). Integrative Sustainability Analysis of European Pig Farms: Development of a Multi-Criteria Assessment Tool. Sustainability, 14(10), 5988. https://doi.org/10.3390/su14105988

The effect of dairy farm extensification under agri-environmental management on nitrogen excretion and farm revenues: A modelling approach

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Introduction: Reducing ammonia emissions is a major challenge for dairy farmers in Flanders and the Netherlands. In the Netherlands, emissions from the dairy sector must be reduced by 40–46%. A substantial share can be achieved through lower dietary nitrogen intake, improved animal management, and technical measures such as air scrubbers and manure processing (Ros et al., 2025). However, these measures alone are unlikely to be sufficient, and extensification of dairy farming near Natura 2000 areas is considered necessary (Ros et al., 2025). Extensification may offer benefits, such as increased plant diversity and improved habitats for meadow birds (e.g. lapwings and godwits) through delayed mowing (Broyer et al., 2016). However, delaying the first cut can strongly affect both the yield and quality of grass silage, with cascading effects on feed intake, milk production, nitrogen excretion, and farm income. This study aimed to simulate the effects of including different proportions of grass silage from a delayed first cut (GLC) in dairy cow rations, and to assess the impact on nitrogen excretion and farm profitability. The results may support the design of financial compensation in agri-environmental schemes (AES).

Material and Methods: Four scenarios (GLC0, GLC20, GLC30, and GLC40) were simulated, in which 0, 20, 30, and 40% of early-cut grass silage (before 15 May) was replaced by late-cut silage (after 15 June), respectively. The baseline scenario (GLC0) represented a 100-cow herd with young stock (0.7/c0w), and in forage supply, with 0.051 ha maize and 0.513 ha grass per cow. Feed intake and milk production were predicted using the Dairy Cow Model (Zom et al., 2012; Zom, 2014) and the Youngstock Model (Mandersloot, 1989). The dry matter yields and feeding values of grass silages were obtained from experimental data of the “Koeien en Kruiden” project (Dairy Campus Leeuwarden, Fryslân; Hoekstra et al., 2023). Diet composition (forage and concentrates) reflected the averages in the North-Western Netherlands (CBS, 2024). The simulated diets were balanced to meet DVE requirements.

The GLC was allocated based on energy (VEM) requirements in the following order: dry cows, young stock (>1 year), and lactating cows. Nitrogen intake and excretion (total N and TAN) were calculated according to Bannink et al. (2020), assuming that 15% of TAN was volatilised as NH_3. Economic outcomes were calculated using standard values from KWIN (2025).

Table 1 Model inputs and assumptions

Herd Demograpy	
Replacement rate	0.28
Young stock/Cow	0.70
Rations (dairy cows, young stock < 1 yo)	
Grazing	180 d, 6h/d
Diet as % Forage DM	
Maize silage	15%
Grass silage	60%
Grazed grass	19%
Wet byproducts	6%
Concentrate kg/cow year	2360

Results and Discussion: Simulation results are presented in Table 3. Delayed cutting reduced dry matter yields, lowering feed availability and consequently reducing herd size and total milk production. In addition, fat- and protein-corrected milk (FPCM) yield per cow decreased with increasing GLC due to lower net energy (VEM) content and higher satiety value (SV; fill value). The combination of fewer cows and a lower nitrogen intake resulted in reductions in TAN excretion of 7%, 9%, and 12% for GLC20, GLC30, and GLC40, respectively, compared to GLC0. This model study suggests that a 10% reduction in ammonia emissions can be achieved when 30–35% of grassland is managed under AES with a delayed first cut. However, this comes at a substantial loss of farm income. Compensation requirements were estimated at €135–225 per kg of NH_3 reduced. Further farm-specific analyses are needed to refine these estimates.

Table 2 Feeding values of concentrates and forages (VEM = net energy for lactation), CP crude protein, DCP digestible CP, DOM= digestible organic matter

	VEM	CP	DCP	DOM
Grass silage early 1st cut[1)]	942	135	88	714
Grass silage delayed 1st cut[1)]	691	79	34	660
Grass silage 2nd and later cuts[1)]	850	151	103	541
Grazed grass[2)]	977	194	146	737
Maize silage[2)]	985	72	31	714
Low protein concentrate	1138	105	73	820
High protein concentrate	1122	288	239	814
Wet byproducts	1062	138	96	746

[1)] Data from Koeien en kruiden (Hoekstra et al. 2023), [2)] Eurofins 5-year average, [3)] CBS, 2024

Table 3 Results of the model simulations on farm system level

% Delayed 1st cut silage	GLC0	GLC20	GLC30	GLC40
Dairy cows (0.7 youngstock/cow)	100	95	93	91
Melkproductie kg FPCM/cow	9481	9473	9349	9224
Total Grass silage intake (t DM)	344	335	326	317
Grass silage delayed 1st cut (t DM)	0	70	105	137
Total area grassland (ha)	51.3	51.3	51.3	51.3
Area delayed 1st cut (ha)	0	9.3	13.9	18.5
Total N intake (kg)	21452	20225	19714	19249
Total TAN-excretion (kg)	8149	7588	7408	7255
NH3-N emission (15% of TAN)	1222	1138	1111	1088
Milk revenues (1000€)	416	396	383	370
Other revenues (1000 €)	59	56	55	54
Feed cost (1000€)	162	160	159	158
Revenues - allocated costs (1000€)	205	194	185	175
Costs per kg NH3-N (€)		134	188	225

References:

Bannink et al. 2020 Animal 14 supp 2 s406

Broyer, et a. 2016 Agr. Ecosyst. Envir. 215, 133.

Hoekstra et al. 2023 Eur. J. Agron. 149, 126886

Mandersloot 1989 PR Rapport 116 https://edepot.wur.nl/28650

KWIN 2025 https://edu.nl/qay6v

Ros et al. Rapport WU 2024.159 https://edepot.wur.nl/684600

Zom et al. 2012 Livestock. Sci 143, 43

Zom 2014 Dairy Cow Model https://edepot.wur.nl/312579

Impact of VEM2022 in the farm specific excretion model BEX for NL

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Introduction: Fertilisation of fields of a dairy farm in the Netherlands is bound by a maximum of 170 kg of Nitrogen (N) from animal sources per year per hectare. Higher production needs to be exported from the farm. N excretion can be calculated using the default values (in Dutch: forfait), for N excretion, which are table values described by the national government. Alternatively, the N excretion of the herd can be proven to be lower by using the 'farm specific excretion– tool' (BEX, in Dutch: Bedrijfsspecifieke EXcretie). In the BEX model farm specific data are used to calculate N excretion for the dairy herd (and other grazing animals). The basis of the BEX model is the estimation of dry matter intake (DMI), which is based on the VEM system. It is assumed that DMI of dairy cows is equal to the VEM requirements +2% divided by the VEM-content of feedstuffs. Up to now the BEX system has been based on the "old" VEM system, however in 2022 CVB has published an update of the VEM system, VEM2022 (CVB, 2025). In order to implement the VEM2022 system as the model to calculate DMI, it is desired to compare it with the old VEM system.

Material and Methods: A database of 294 year observations of on farm measured dry matter intake from the 'cows and opportunities-project' (In Dutch: koeien en kansen) for the period of 1999-2021 was used to evaluate and calibrate estimated DMI to measured DMI farm data. On farm, DMI was measured in multiple so called 'measurement weeks', which were averaged to give one value for the average DMI of the cows on the every farm. After calibration of the predicted DMI the VEM2022 model was implemented in the BEX model to calculate farm N-excretion of 984 anonymised real live farms, representative for the Netherlands derived from the Dutch Zuivel NL database.

Results and Discussion: Measured DMI in the 'cows and opportunities'- project was 2.8% and 5.5% higher than estimated with, the VEM and VEM2022 system respectively. The 2.8% bias is close to the currently used correction value of 2% for the VEM system to calculate DMI. VEM2022 will need a 5.5% bias correction for implementation in BEX. This bias was not constant during the years and increased in time. This effect is currently not understood. Using a 5.5 % bias corrected DMI estimation with VEM2022 the effect on N excretion was compared with the current BEX N-excretion. In the 984 farm database with an average milk yield of 9559 kg FPCM per year, implementation of VEM2022 relative to VEM on average reduced N excretion by 1.4%, however for individual farms the change varied form -7.3 to +7.9% N excretion.

This range was highly correlated with the average on farm milk yield, where low milk yield resulted in a higher N excretion and high milk yield resulted in a lower N excretion than calculated with the VEM-system. This is consistent with the background of the VEM2022 system, as maintenance requirements are increased but the utilization of energy for milk yield is evaluated to be more efficient than in the former period where the VEM-system was developed.

References: CVB, 2025, Geactualiseerde energiebehoeftenormen voor Holstein Friesian melkkoeien versie 2025. CVB documentatierapport nr. 79. (https://www.cvbdiervoeding.nl/pagina/10081/downloads.aspx); Handreiking bedrijfsspecifieke excretie melkvee. (https://www.rvo.nl/sites/default/files/2025-04/250402%20Handreiking%20BEX%202025%20versie%201.0.pdf)

Enhancing the fiber degradation efficiency in dairy cattle rumen through engineered bacterial communities

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Introduction: The rumen functions as an anaerobic fermentation chamber, housing microorganisms with cellulolytic and proteolytic capabilities that facilitate feed utilization. Fiber-degrading bacteria possess the capability to enhance the productivity of cellulolytic feed. The application of omics technologies has greatly improved our understanding of the rumen microbiome. Determining microbial composition and functional patterns in the rumen does not equate to a comprehensive exploration of rumen microbial resources and their mechanisms of action. This study seeks to integrate high throughput 16S rRNA data with information on culturomics, cellulolytic activities, nutrition, and synthetic microbial communities (SynCom) engineering. The objective is to evaluate the relationship between rumen microbial activity and fiber utilization efficiency in cattle, ultimately aiming to develop a more powerful intervention strategy for the ruminant industry.

Material and Methods: The enrichment culture with various carbon sources led to significant alterations in the composition and structure of rumen microbiota, particularly enhancing those associated with carbohydrate metabolism. Employing the culturomics methodology, 896 strains from 78 species (including 8 novel species) were isolated, resulting in a 10.1% isolation rate relative to the rumen bacterial community. Among them, 35 strains demonstrated boosted cellulose-degrading capability on plates, while 25 exhibited the ability to degrade hemicellulose as well. SynComs of these candidates were prepared based on the ratio observed in rumen microbiota exhibiting high cellulolytic performance. SynCom 3 improved the neutral detergent fiber degradation (NDFD) by 20.39% averagely. Additionally, both in vitro and in situ assessments indicated that the optimization of dose/strain in SynCom 3 significantly improved the in vitro NDFD by 20.56% and increased the in situ NDFD by 7.81%, along with the acidic detergent fiber (ADF, + 11.47%). Genomic analysis revealed that the SynCom 3 functioned well in fiber degradation through the synergistic action of key carbohydrate-active enzymes.

Results and Discussion: This study strengthens rumen microbiome research by integrating omics and SynCom engineering within a microbiota-bacteria-enzymes-genes framework, revealing the significance of enzymatic synergy in carbohydrate metabolism. The findings establish a framework for utilizing low-abundance microbes and engineering functional consortia, which are crucial for improving ruminant feed utilization and biomass conversion. Future research should investigate the transcriptomic profiles and the metabolic cross-feeding mechanisms of fiber-degrading strains in the rumen

Nutritional evaluation a forage quality gradient: Validation of Forage Quality Score (FQS) through rumen dynamics, nutrient digestibility, and energy retention in beef cattle

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Abstract

This study evaluated the relationship between the Forage Quality Score (FQS, 0-100), a comprehensive quality index derived from crude protein (CP), ash free neutral detergent fiber (NDF), and 48 h NDF digestibility and the *in-situ* degradability, rumen fermentation, nutrient digestibility, nitrogen balance, blood biochemistry, and antioxidant status of beef cattle fed forage-based diets. Seven ruminally cannulated beef cattle (4 bulls, 3 heifers) were used in a partially replicated 5 × 5 Latin square design. Treatments included alfalfa silage (FQS: 69) and four mixtures (alfalfa or *Sesbania cannabina* with oats or ryegrass; 35:65 DM ratio) with FQS ranging from 38 to 63, all fed at a 45:55 concentrate-to-forage ratio. Ruminal degradability was estimated using *in-situ* tests. Fermentation parameters were analyzed from rumen fluid collected at 0, 1, 2, 4, 6, and 8 h post-feeding. Nutrient intake, nutrient digestibility, and N balance were determined via total fecal and urinary collection. *In-situ* effective degradability (ED) and degradation rates (c) of DM and NDF increased linearly with FQS ($P < 0.001$), though CP degradability remained unaffected. Intake of organic matter (OM), CP and aNDFom increased linearly with FQS ($P = 0.05$). Each 1-unit increase in FQS was associated with a 0.38% unit increase in OM digestibility ($P = 0.034$) and linear increases in N retention ($P < 0.001$) and ME/GE ($p = 0.001$). Forage Quality Score affected negatively ruminal pH at 1 h post-feeding ($P < 0.001$). Ammonia-N increased linearly ($P < 0.001$) as FQS decreased across all time points. Microbial crude protein (MCP) showed no differences at 0 and 8 h but increased linearly with FQS at 1 and 2 h post-feeding.

Most plasma parameters remained stable across treatments ($P > 0.05$), including glucose, protein fractions (total protein, albumin, and globulin), blood urea nitrogen (BUN), lipid profiles (total cholesterol and triglycerides), total antioxidant capacity (T-AOC), and glutathione peroxidase (GSH-PX). Notably, plasma superoxide dismutase (SOD) activity exhibited a significant linear increase ($P < 0.001$) in response to improved dietary FQS. In conclusion, the FQS proved effective for predicting ruminal degradability, nutrient intake, digestibility, and N utilization in beef cattle, proving a validated tool for precision forage evaluation.

Key words: forage, intake, degradation, digestibility, score, quality.

Influence of inoculum type and substrate on the efficacy of 3-nitrooxypropanol in inhibiting methane production in vitro

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Introduction: Enteric methane (CH_4) production from ruminants contributes to greenhouse gas emissions and represents a loss of dietary energy (Gerber et al., 2013). The feed additive 3-nitrooxypropanol (3-NOP) inhibits methanogenesis by targeting methyl-coenzyme M reductase in methanogenic archaea (Duin et al., 2016). However, the magnitude of CH_4 mitigation can vary across feeding conditions and experimental systems (Kebreab et al., 2023). Part of this variability may be related to differences in rumen fermentation patterns and microbial community characteristics, which influence hydrogen (H_2) utilization pathways and the competition between methanogenesis and alternative H_2 sinks such as propionate formation (Ungerfeld, 2020). Nevertheless, it remains unclear whether feed type or rumen microbial composition plays a dominant role in determining the efficacy of 3-NOP. Therefore, the objective of this study was to determine whether differences in CH_4 mitigation efficacy of 3-NOP are driven by microbial inoculum characteristics or by substrate composition in an in vitro rumen fermentation system.

Material and Methods: In vitro batch incubations were conducted using rumen inocula collected from six donor cows. Three cows adapted for at least 3 weeks to a standard-starch diet (18.0% starch) and three to a high-starch diet (26.8% starch), providing the standard-starch inoculum (SI) and the high-starch inoculum (HI), respectively. The experiment followed a factorial design including three donor cows within each inoculum type, two inoculum types, two substrate types and two treatments (control or 3-NOP at 72 mg/kg DM). The experiment was performed over 6 runs (SI and HI, 3 runs each), with triplicates per treatment within each run. The two substrates corresponded to the same dietary starch levels used for inoculum adaptation and were referred to as standard-starch substrate (SS) and high-starch substrate (HS). CH_4 production and total volatile fatty acids (VFA) were measured after 24 h of incubation, and methane yield and CH_4/VFA were calculated. Data was analyzed using mixed-effects models including run and donor cow as random effects.

Results and Discussion: 3-NOP significantly reduced CH_4 production in both inoculum types ($P < 0.001$; Table 1), with a stronger reduction observed in the high-starch inoculum. Total VFA production was not affected by 3-NOP, although a significant 3-NOP x substrate interaction was observed in the SI group ($P = 0.025$), but not in the HI group, indicating that overall fermentation activity was maintained and the response depended on substrate type within SI inocula, but this effect was not consistent across inocula types. However, the CH_4/VFA ratio decreased markedly, suggesting a redirection of reducing equivalents away from methanogenesis towards alternative fermentation pathways.

No significant substrate x 3-NOP was observed. The differences between inocula therefore likely reflect microbial community characteristics established in the donor animals through dietary adaptation. High-starch diets typically favor propionate-producing bacteria, which provide alternative hydrogen sinks and may enhance the response to methanogenesis inhibition. The absence of a substrate effect in vitro may reflect limitations of the incubation system, where buffered pH and short incubation periods do not allow microbial populations to adapt to the substrates as occurs in vivo.

Table 1 Effects of 3-NOP and substrate on fermentation responses using standard-starch and high-starch inocula (CH_4: mmol g^{-1} DM; VFA: mmol g^{-1} DM; CH_4/VFA: mmol mol^{-1})

Response variable	Intercept	3-NOP	Subs	3-NOP x Subs.	*P*-value			
					Intercept	3-NOP	Subs.	3-NOP x Subs.
Selected mixed model: Response variables = 3-NOP + Subs + 3-NOP x Subs + random(Run + Cows).								
Standard-starch inoculum								
Total VFA	5.73 ±0.16	-0.18 ±0.13	-0.14 ±0.13	-0.41 ±0.18	<0.001	0.749	0.463	**0.025**
Total CH_4	1.77 ±0.12	-0.33 ±0.08	-0.001 ±0.077	-0.03 ±0.11	<0.001	**<0.001**	0.834	0.823
CH_4/ VFA	304 ±34.3	-66 ±24.0	4 ±23.5	-0.01 ±33.82	<0.001	**<0.001**	0.816	1.000
High-starch inoculum								
Total VFA	5.89 ±0.26	-0.03 ±0.12	0.10 ±0.12	0.04 ±0.16	<0.001	0.875	0.160	0.825
Total CH_4	1.70 ±0.19	-0.70 ±0.07	-0.08 ±0.07	0.06 ±0.11	0.011	**<0.001**	0.362	0.599
CH_4/ VFA	265 ±29.4	-97 ±19.9	-11 ±19.2	-3 ±27.8	0.010	**<0.001**	0.378	0.914

Notes: Values are estimated marginal means (Estimate) ± standard error (SE) obtained from the mixed-effects model, and the statistical significance was considered at $P \leq 0.05$.

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Efficacy of 3-NOP in mitigating CH_4 production in relation to the chemical composition of the in vitro substrate

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Introduction: Enteric methane emissions from cattle contribute to global warming. To accelerate the implementation of methane mitigating feed strategies for cattle, assessment of antimethanogenic feed additives need to be optimized. An integrated and validated in vitro – in vivo approach could lead to a more rapid assessment of the reduction potential of new measures. This study investigated the relationship between the in vitro efficacy of the antimethanogenic compound 3-nitrooxypropanol (3-NOP) and the chemical composition of the in vitro substrate and explored if the known interaction from in vivo studies can also be observed in vitro (Kebreab et al., 2023). We hypothesized that the effect of 3-NOP would be enhanced by a higher dietary starch content. By (quantitatively) comparing the variation in the mitigating effect of 3-NOP observed in vitro with the variation associated with the basal diet in vivo, the in vitro set-up can be validated, making in vitro screening and extrapolation more robust.

Material and Methods: In vitro fermentation was investigated in 2 batch culture experiments in which 2 substrates, a standard-starch (SS: 179 g starch/kg DM; 362 g NDF/kg DM) and high-starch (HS: 270 g starch/kg DM; 302 mg NDF/kg DM) substrate, were incubated using pooled inoculum from 3 rumen- fistulated Holstein dairy cows either fully adapted (>21 d) to SS (experiment 1) or to HS (experiment 2). Both substrates were supplemented with a control (0 mg 3-NOP/kg DM) and 3-NOP (72 mg 3-NOP/kg DM) treatment. Each experiment consisted of 3 in vitro runs (with a one-week interval), resulting in two 2x2 randomized complete block design experiments (with run as a blocking factor). Gas production was measured at various incubation times (2, 4, 6, 8, 24, 32 and 48 h). CH_4 production (mg/g incubated DM) data were fitted by:

$$CH_4\ production = \frac{A}{1+\left(\frac{B}{t}\right)^C} \quad (1)$$

(Groot et al., 1996), where A represents the asymptote of methane production (mg/g incubated DM); B is the incubation time (h) at which half of maximum methane production has been formed, and C defines the sharpness of the switching characteristic for the profile (unitless), where the profile has an inflection point when C > 1.

The rate of CH_4 production (mg/g incubated DM/h) was calculated as the first derivative of equation 1 and thus fitted by:

$$CH_4\ production\ rate = \frac{AB^C C}{t^{C+1}\left(1+\left(\frac{B}{t}\right)^C\right)^2}. \quad (2)$$

The time (h) of maximal CH_4 production rate (tR_{max}) was determined by:

$$t_{R_{max}} = B\left(\frac{C-1}{C+1}\right)^{\frac{1}{C}}, \quad (3)$$

and the maximal CH_4 production rate (R_{max}) was calculated by replacing t with tR_{max} in equation 2. Methane and associated kinetics data were analyzed by ANOVA using linear mixed modelling (Y ~ SUB*TRT + (1|RUN)) per experiment. Treatment means were expressed as estimated marginal means, and the statistical significance was considered at $P \leq 0.05$, and a trend at $0.05 \leq P \leq 0.10$.

Results and Discussion: In contrast to the hypothesis, absolute (CH_4 48h) and relative (CH_4/total VFA 48h) methane production, and the kinetics parameters (A, B, C, tR_{max}, and R_{max}) related to absolute CH_4 production in experiment 1 (inoculum adapted to SS) and 2 (inoculum adapted to HS) were not affected ($P \geq 0.075$ and $P \geq 0.246$, resp.) by the interaction between the substrate composition and the addition of 3-NOP. Besides the maximum CH_4 production rate (R_{max}) in experiment 2 ($P = 0{,}049$), methane production and its associated kinetics parameters did not differ ($P \geq 0{,}106$) across substrates in experiment 1 and 2. In experiment 1, the switching characteristic of the CH_4 production curve (C), the time at which R_{max} occurred (tR_{max}), and R_{max} were affected ($P = 0.049$, $P < 0.001$, and $P = 0.008$, resp.) by the 3-NOP treatment, where 3-NOP led to more sigmoidal profiles (Figure1(A)). In experiment 2, absolute CH_4 production and all associated kinetics parameters were influenced ($P \leq 0.033$) by the addition of 3-NOP, while the CH_4/total VFA ratio tended to be reduced ($P = 0.051$) by the 3-NOP treatment. The known degradation of 3-NOP in the rumen is indicated by the decreasing effect of 3-NOP over time in both experiments (Figure 1). With the set-up and experimental design used, the known in vivo interaction between the dietary chemical composition and efficacy of 3-NOP could not be observed in vitro. A larger contrast in starch content between the substrates might be necessary to compensate for the strong differences between in vitro and in vivo conditions.

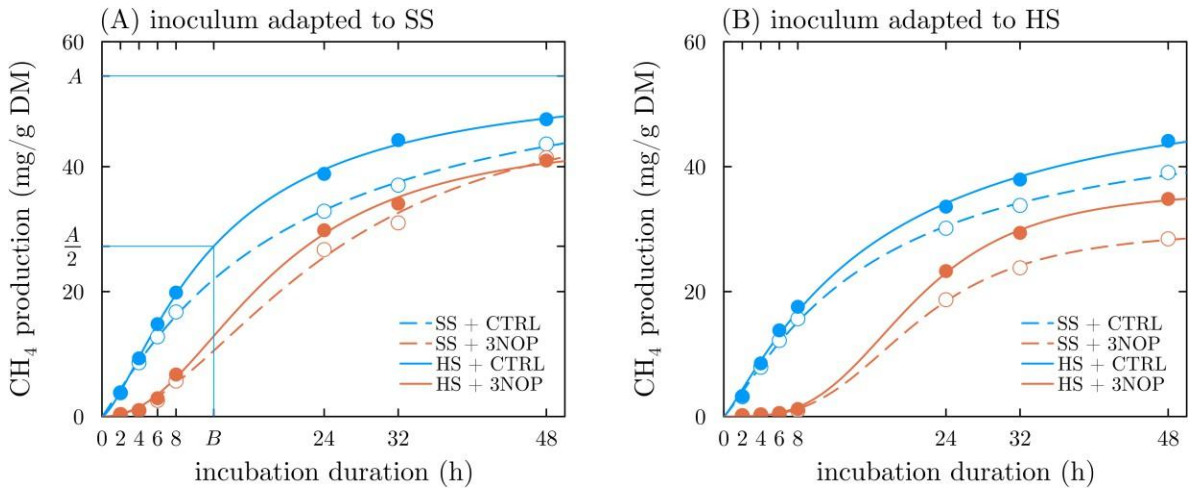

Figure 1: The cumulative methane production(mg/g incubated DM) curve described by equation 1 (Groot et al., 1996), with A: asymptote of methane production (mg/g incubated DM); B: incubation time (h) at which half of maximum methane production has been formed; C: the sharpness of the switching characteristic for the profile (unitless). (A) Experiment 1 (inoculum adapted to SS), with indications of A, A/2, and B for HS + CTRL; (B) Experiment 2 (inoculum adapted to HS). Observed CH_4 production data at distinct incubation times are indicated by circles (∘).

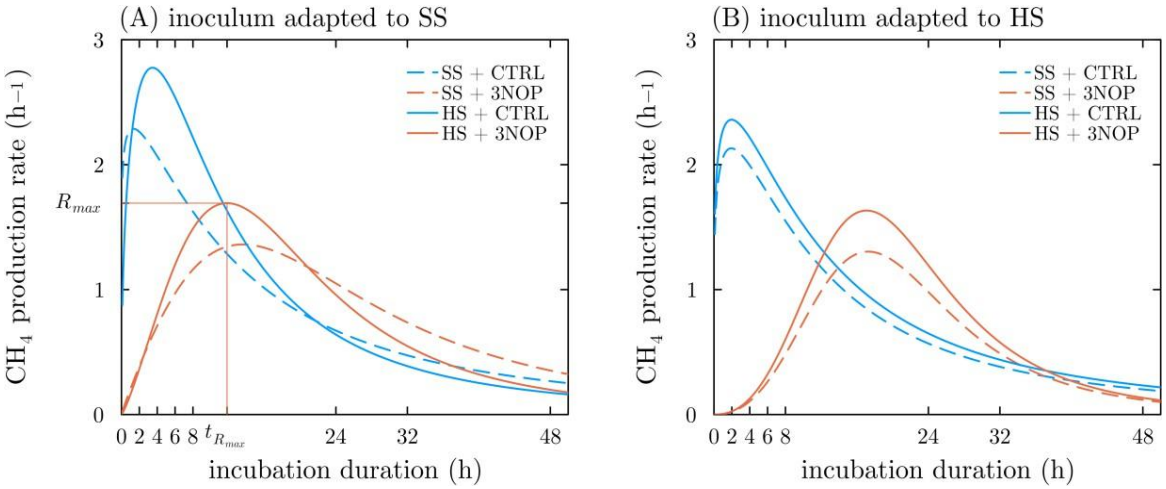

Figure 2: Methane production rate (mg/g incubated DM/h) described by equation 2, the first derivative of the model described by Groot et al. (1996) (Figure 1), with A: asymptote of methane production (mg/g incubated DM); B: incubation time (h) at which half of maximum methane production has been formed; C: the sharpness of the switching characteristic for the profile (unitless). (A) Experiment 1 (inoculum adapted to SS), with indications of tR_{max} and R_{max} for HS + 3NOP; (B) Experiment 2 (inoculum adapted to HS).

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SESSION 2

Dietary characteristics affect enteric methane and odour formation in pigs: insights from colon digesta incubation and analysis of exhaled air

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Abstract

Reducing gaseous emissions from pig production is important for mitigating environmental impact and improving barn-air quality. Fermentation processes in the large intestine contribute to emissions through microbial degradation of endogenous and unabsorbed dietary components, leading to the formation of gases such as CH_4 and odorous compounds. This study investigated how dietary protein, sulphur and fibre influence intestinal fermentation and associated gas production in pigs.

Thirty-six pigs were distributed over six dietary treatments in a 3x2 factorial design consisting of three crude protein-sulphur combinations (highP-highS, highP-lowS, lowP-lowS), each with and without fermentable (inulin) and insoluble fibre (oat hulls). Methane concentrations in exhaled air were measured in sedated pigs using FTIR spectroscopy. After euthanasia, proximal colon digesta samples were collected and incubated for 24h to quantify CH_4 and total gas production. After 24h, headspace samples were analysed for odorous compounds composition using GC-MS and GC-SCD.

Both total gas and CH_4 production (mL/g OM) were lower for high-fibre compared with low-fibre diets. Methane production was lower for highP-highS compared with lowP-lowS diets. Exhaled air CH_4 concentration was positively correlated with total CH_4 production during colon digesta incubation (r=0.693). Differences in odour-related compounds between treatments were relatively small.

These findings show that dietary fibre can influence colon fermentation processes linked to enteric CH_4 and odour formation, highlighting the potential of dietary strategies to mitigate emissions in pig production. The relationship between CH_4 in exhaled air and colon fermentation indicates that breath analysis may provide a promising tool to investigate enteric CH_4 formation in pigs.

The Effect of Choice-Feeding between High- versus Low- Crude Protein Diet on Feeding Behaviour in Weaned Piglet

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Introduction: Choice feeding allows individual animals to self-select dietary protein intake, but whether dietary differentiation reflects voluntary appetite or competitive feeder access remains unclear. This study applied Hidden Markov Model (HMM) to characterise individual feeding behaviour in weaned piglets offered a choice between high-crude protein (HCP) and low-CP (LCP) diets.

Material and Methods: A total of 100 weaned piglets, aged 26-28 days, were included in the study. At weaning (day 0), piglets had an average body weight (BW) of 6.25 ± 0.33 kg (mean ± SEM). Piglets were randomly allocated to five pens (20 piglets per pen) while ensuring a balanced distribution of sex and BW across pens. To evaluate voluntary diet selection, two diets differing in crude protein content were simultaneously offered within each pen across 42 days post-weaning: the HCP diet containing 190 g/kg crude protein and 12.0 g/kg standardized ileal digestible (SID) lysine, and the LCP diet containing 160 g/kg CP and 12.1 g/kg SID lysine. Individual feeder visit data were used to derive two features per visit: diet choice and a net social competition index. The daily posterior probabilities of each state and state occupancy rates were computed from per pig using an HMM Piecewise linear regression with a fixed breakpoint was applied to identify a behavioural changepoint.

Results and Discussion: The HMM identified three behavioural states, including HCP-persistent, Opportunistic, and LCP-persistent. Population-level HMM output showed HCP-persistent state dominance during d0-14 shifting to Opportunistic and/or LCP-persistent dominance post-d14. Piecewise regression on daily HCP posterior probability, posterior entropy, and switching rate consistently identified d14 as a behavioural changepoint, supported by a significant reduction in within-pig HP posterior variability (CV: 0.63 pre-d14 vs 0.52 post-d14; Wilcoxon $P = 0.002$) and a decline in the net social competition (0.062 vs. 0.035; Wilcoxon $P < 0.001$).

Conditioning feed influences starch digestion kinetics in an ingredient dependent manner

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Introduction: Feed manufacturing processes are crucial in improving the nutritional properties of feed ingredients and complete feeds. The machines used in the processes apply thermal and mechanical energy to feed mixtures resulting in changes in rate and degree of nutrient digestion. Applying energy along with moisture during the steam and pressure conditioning processes causes loss of crystalline structure of starch (i.e. gelatinization). This results in increased interaction between starch degrading enzymes and starch, influencing the starch digestion kinetics (SDK). However, the relationship of conditioning intensity/energy input on degree of starch gelatinization and SDK is still understudied but its understanding would be instrumental for tuning feed processing to steer the nutrient release and energy value.

Material and Methods: In our study, experimental feeds based on maize, wheat and barley (cereals), faba bean (legume), wheat middlings and oat hulls (co-products) were prepared at different conditioning intensities/energy inputs. Conditioning was done at specific thermal energy in a steam conditioner (STEc) at 60, 70, and 80 °C and then exposed to low (L≈10 kWh/t) or high (H≈20 kWh/t) specific mechanical energy in an expander (SMEe), except for the 70°C treatment. Following this, all feed mixtures were pelleted in a pelletizer (SMEp). SDK of the experimental feeds were analyzed using an *in vitro* digestion system and estimation of fractional rate (k) and plateau (extent) by non-linear regression using a modified Chapman Richard's model. The degree of starch gelatinization (SGD) of feeds were estimated by enzymatic method and differential scanning calorimetry. GLM model with weighted least square regression was used to assess the impact of energy and SGD on rate and plateau of starch digestion.

Results and Discussion: The k values increased with increase in total energy input (STEc + SMEe + SMEp) ($p<0.001$). The highest k value was observed at the highest conditioning intensity (80HP: 2.4-7.2%/min), whereas the lowest k occurred at lowest intensity (70P: 1.7-3.0%/min).

The increase in k with the increase in energy within a starch source may be due to high degree of gelatinization (Svihus et al., 2005; Mahasukhonthachat et al., 2010) and it is explained by the correlation between SGD and k ($R^2=0.96$, $p<0.001$). Effects of conditioning intensity on k were ingredient-specific ($p<0.001$). The increase in k with increase in energy input was highest in faba bean-based diets and lowest in maize-based diets. The difference in responses of different ingredients to energy input can be attributed to variations in their starch structure and properties (Dhital et al., 2017; Martens et al., 2018). Plateau values were not affected by the conditioning intensity ($p>0.05$).

The findings of the study indicate the possibility of developing ingredient-specific conditioning strategies to optimize SDK and machine energy use in feed manufacturing. Further, SGD can be used as a reliable predictor of SDK.

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Zootechnical and physiological impacts of adding a concentrated solution of Chlorella vulgaris FAM28093 to feed for post-weaning piglets: a pilot study

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Introduction: Microalgae are increasingly explored as alternative feed ingredients in pig production due to their nutritional value and sustainability potential (Kurpan et al., 2025). However, their application in practice remains limited, partly because of processing constraints. In the present study, we evaluated the effects of including a minimally processed, liquid microalga supplement in the diet of post-weaning piglets, focusing on feed acceptance, growth performance, and digestive outcomes.

Material and Methods: A wet feed supplemented with Chlorella vulgaris was produced by incorporating a concentrated microalgal solution (4% DM) into a standard piglet diet in a 1:1 ration. Feed characteristics were first assessed through aroma profiling and in vitro protein digestibility. Subsequently, 24 post-weaning piglets (initial body weight 18.1 ± 3.55 kg) were fed either a control diet or the microalga-supplemented diet for two weeks. Body weight gain, feed intake, fecal dry matter and total bacteria output were recorded.

Results and Discussion: In vitro analyses showed a 10% increase in protein digestibility of the microalga-supplemented feed compared to the control. Although the inclusion of microalgae modified the feed aroma profile, this did not negatively affect feed acceptance in vivo. Piglets fed the microalga-supplemented diet exhibited similar ($p > 0.05$) daily weight gain (0.83 ± 0.17 kg d^{-1}) and feed intake (6.00 ± 0.66 kg d^{-1}) compared with control animals. Fecal dry matter content was significantly higher in piglets receiving the microalga-supplemented diet (214 ± 18.1 g kg^{-1} vs. 188 ± 11.8 g kg^{-1}; $p < 0.001$), indicating an effect on digestive output without compromising growth performance. Overall, these results demonstrate that liquid Chlorella vulgaris can be successfully included in piglet diets without negative effects on performance or feed acceptance. This approach offers a promising strategy for integrating microalgae into pig production systems while maintaining animal performance.

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Utilizing waste streams for earthworm production as a protein source: impact of substrate composition on growth and body nutrients.

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Introduction: In countries such as Ethiopia, access to protein-rich feed resources remains a major challenge. Dependence on imported feeds often results in unsustainable economic, social, and environmental outcomes, while the use of locally available resources, such as waste substrates, is constrained by their low protein content (1). Therefore, there is a need to explore alternative approaches to convert low-protein substrates into high-protein biomass. Earthworms represent a promising solution in this regard. However, limited information is available on how substrate composition influences the growth performance and nutrient profile of earthworms. Consequently, this study evaluated the effects of substrates with varying protein levels on the performance and nutrient composition of *Eisenia fetida*.

Material and Methods: Two mixed substrates with different protein concentrations were formulated using locally available waste materials, based on substrate preference tests and availability. Treatment one (T1) consisted of cow manure, rumen liquor, fruit leftovers, and moringa leaves, whereas treatment two (T2) included cow manure, rumen liquor, fruit leftovers, and grass. The two experimental substrates were evaluated at three time points (day 20, day 40, and day 60), with five replicates at each time point. All experimental substrates were mixed at a volume ratio of 1:1:1:1 on a dry matter basis in which soil and coffee chaff, mixed at a volume ratio of 2:1 (dry basis) was used as bedding material. After mixing, water was gradually added until optimal moisture conditions were achieved. The prepared substrate mixtures were transferred into 40-L plastic containers and pre-composted for seven days prior to earthworm introduction. Subsequently, 200 earthworm larvae were introduced into each experimental unit. During the experiment, moisture, temperature and pH were monitored. Worms were collected at the end of each time point and processed for the analysis of protein and fatty acid profiles. Results were analysed using repeated measures analysis in SPSS.

Results and Discussion: Overall, the protein concentrations were high, ranging from 51.3% to 58.2%. The highest protein content (58.8 ± 2.4%) was recorded in earthworms from T1 on day 20, followed by day 60 (58.2 ± 2.0%).

Protein levels in earthworms from T2 were significantly lower than T1, with 51.3 ± 1.5% on day 40 and 54.95 ± 0.8% on day 60 ($P < 0.05$). The higher protein content in T1 may be attributed to the greater protein content of its substrate combination. The worms also exhibited a favourable fatty acid profile, being rich in EPA (C20:5n-3, 2.56%–3.99%), with ARA (C20:4n-6) dominating across treatments (4.33%–5.7%). Linoleic acid (C18:2n-6) was present in adequate amounts (4.38%–5.79%), whereas DHA (C22:6n-3) levels were low. The n-6:n-3 ratio was 2.4 in T1 and 2.9 in T2, indicating a more desirable fatty acid profile in Treatment 1.

Overall, earthworms performed well in terms of protein accumulation even when cultured on low-protein waste substrates and a substrate combining fruit waste, rumen liquor, and cow manure. Despite their responsiveness to dietary protein, earthworms can yield high-protein biomass on low-protein substrates, offering a sustainable and cost-effective approach to feed production.

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Black Soldier Fly Larvae-Derived Chitin and Chitosan as a Strategy against Enterotoxigenic *Escherichia coli*-Infections in Piglets: an *In vitro* Study

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Introduction: Post-weaning diarrhea (PWD) caused by enterotoxigenic *Escherichia coli* (ETEC) predominantly affects newly weaned piglets. As piglets are prematurely weaned, they become particularly vulnerable due to their immature gut physiology, microbiome, and immune system. Following the ban on antibiotic growth promoters and zinc oxide, ETEC-induced PWD remains a challenge in pig production. Black soldier fly larvae (*Hermetia illucens*, BSFL) are rich in chitin, which can be deacetylated to form chitosan. Both substances possess antimicrobial and immunomodulatory properties, representing a potential solution against ETEC-infections[1]. The objective of this study is to evaluate their effects during an ETEC challenge, using a simulated gut model combining static *in vitro* digestion with ETEC-infected *in vitro* batch fermentation.

Material and Methods: Post-weaning pig feed was ground, sieved through 1 mm, and supplemented with 1% additives to create six treatments: control, commercial chitin, BSFL-derived chitin, commercial chitosan, BSFL-derived chitosan, and inulin (positive control). Static INFOGEST *in vitro* digestion, modified for young piglets, was performed in triplicate. For the gastric phase, pepsin (140 U/mL) was added at pH

3.0 and incubated for 2 h at 39°C in a shaking hot water bath. For the intestinal phase, the pH was adjusted to 7.0, and α-amylase (200 U/mL), trypsin (30 U/mL), and chymotrypsin (7.5 U/mL) were added and incubated for 4 h at 39°C. An aliquot of the digested samples was used to determine the degree of protein hydrolysis (DPH) via an o-phthalaldehyde assay after 5% TCA precipitation in triplicate. The remaining digested samples underwent *in vitro* absorption using a 3.5 kDa membrane in 1× PBS for 72 h at 4°C.The non-absorbed fraction was freeze-dried and diluted in Menke's buffer with jejunal inoculum from piglets two weeks after weaning (1:9 ratio jejunal content:buffer), prepared under anaerobic conditions for *in vitro* batch fermentation at 39°C in quadruplicate. Gas production was measured at 2, 5, 8, 12, 16, 20, and 24 h, and samples were collected at 6, 12, and 24 h. To determine the optimal ETEC dose for subsequent ETEC-infection fermentation, a preliminary experiment was performed in triplicate using only pig feed and inoculum spiked with ETEC at three doses: high (non-diluted), medium (10^{-2} dilution), and low (10^{-4} dilution). Fermentation conditions were identical to those described above. Data were analyzed using non-parametric Kruskal-Wallis test, with significance set at $P < 0.05$.

Results and Discussion: The chitosan and chitin additives had no significant effect on DPH. In the non infected fermentation, although not statistically significant, commercial chitin and chitosan showed a higher final cumulative gas production than the control, with chitin showing higher gas production than chitosan. BSFL-derived chitin and chitosan showed a lower final gas production than the control, with chitosan producing more gas than chitin. Inulin showed an intermediate gas production.

In the ETEC- challenged fermentation, the low ETEC level produced the highest final cumulative gas production compared to the medium and high inoculation levels, which reached approximately equal volumes ($P > 0.05$). Next, we will analyze the short chain fatty acids production in both the non-infected and ETEC- infection fermentations. Further, the ETEC abundance will be quantified via qPCR at different timepoints for both the preliminary and ETEC-infection fermentations. Additionally, the gas production will be measured in the ETEC-infection fermentation with all treatments.

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The potential of bile acids in avian metabolism.

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Introduction: Mammals maintain a closed enterohepatic circulation (EHC) between the intestines and the liver, with limited bile acid (BA) levels outside this system. In contrast, it has been hypothesized that birds exhibit a more open enterohepatic circulation. Preliminary data indicate that birds have high concentrations of conjugated BAs in both portal and peripheral blood. Such a system could support the exceptionally high reliance on fat metabolism observed in birds. Beyond their role in lipid digestion, the physiological function, distribution, and composition of BA in poultry remain insufficiently understood. We hypothesize that circulating BAs contribute to systemic lipid transport and energy regulation depending on the diet and genetics, while low levels of secondary BAs reflect microbiome interactions that protect the systemic circulation from their accumulation.

Materials and methods: As a first step, we explored BA metabolism in broiler chickens and laying hens in response to different diets. Two experiments were conducted. The first one was a digestibility study in both genotypes fed either a highly digestible diet based on corn and soy oil or a challenge diet based on wheat, rapeseed meal and palm oil, to assess effects on fat digestibility and BA excretion. In the second descriptive experiment with broilers, BAs were measured in multiple matrices with LCMS-MS, including the gall bladder, intestinal contents, and mesenteric and peripheral plasma pools. Using Ti02 as indigestible marker digestibility coefficients of multiple BAs were calculated between duodenum and colon.

Results and discussion: In broilers, the highly digestible diet increased plasma triglycerides and non-esterified fatty acids pointing towards efficient uptake of dietary fat, however laying hens were less responsive of the diet-type. BA excretion correlated weakly with digestibility in both breeds. All pools were dominated by (tauro)chenodeoxycholic acid and taurocholic acid. Intestinal reabsorption of conjugated BAs was more efficient than that of deconjugated BAs. BA concentrations in peripheral circulation were similar to those in the mesenteric veins with a high conjugation ratio in comparison with mammalian studies. Overall, BAs appear to circulate more openly in the peripheral circulation, indicating that avian BA metabolism may be more complex than in mammals and cannot, on its own, explain diet-induced differences in fat digestibility if there are any.

Table 1. Effect of diet type (corn-soy based diet (CS) or wheat-palm oil-rapeseed based diet (WRP)) on fecal digestibility coefficients (%) of crude fat (aDCCF), plasma non-esterified fatty acids (NEFA, mg/dL), plasma triglycerides (TG, mg/dL), total bile salt content (µg/g FM) and total daily lost (mg) in excreta of male finisher broilers (25 days) and laying hens (32 weeks) (broiler n=18; layer n=12).

	broiler		*P*	layer		*P*
	CS	WRP		CS	WRP	
aDCCF	84.11[a]±3.42	54.66[b]±7.54	<0.001	85.40±2.86	83.05±4.48	0.130
NEFA	0.78[a]±0.18	0.38[b]±0.09	<0.001	0.26±0.08	0.29±0.09	0.328
TG	167[a]±28	104[b]±35	<0.001	1817±846	1761±402	0.842
Total BA	398.66±122.60	383.02±75.74	0.646	323.03±101.94	437.23±230.08	0.117
Total BA lost	45.71±12.73	54.04±13.60	0.058	30.83±10.46	41.70±24.90	0.174

Data represents arithmetic means ± 1SD
[a,b] superscripts represent significant pairwise post-hoc comparisons ($p<0.05$)

Table 2. Total bile salt content, digestibility coefficients (%) and conjugated BA ratios in intestines, blood or bile of male finisher broilers (day 39) (n=12).

Duodenum		
	Total (mg/gDM)	19.42±9.40
	Conjugated (%)	90.09±4.76
Colon		
	Total (mg/ gDM)	1.63±0.94
	Conjugated (%)	12.73±4.27
	dTCA (%)	99.86±0.14
	dTCDCA (%)	99.61±0.34
	dCA (%)	96.15±4.81
	dCDCA (%)	78.47±20.06
mesenterial blood		
	Total (µM)	25.56±13.32
	Conjugated (%)	93.98±2.65
Peripheral blood		
	Total (µM)	11.23±5.73
	Conjugated (%)	85.85±7.68
Bile[x]		
	Total (mg/gDM)	508.11±44.78

[x]All BAs in the bile are conjugated.
Data represents arithmetic means ± 1SD
d(T)CA digesiblity coefficient of (tauro)cholic acid
d(T)CDCA digestibility coefficient of (tauro)chenodeoxycholic acid

SESSION 3

Interactive effects of dietary tryptophan and large neutral amino acids on growth performance and behaviours in weaned piglets

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Introduction: Stress-related behaviours are the primary welfare concerns in the pig industry. Nutritional strategies may potentially decrease and prevent these behaviours while maintaining or improving growth performance. Tryptophan (Trp) serves as a precursor of serotonin in the brain, which plays an important role in regulating mood, stress response, and behaviour. However, the availability of Trp for serotonin synthesis can be influenced by large neutral amino acids (LNAA), which compete with Trp to cross the blood–brain barrier by using the same transporter. Therefore, the balance between dietary Trp and LNAA may affect piglets' behaviour and indirectly influence performance through changes in appetite and stress regulation. This study examined optimal dietary Trp levels to reduce bite-related damage and aggression in weaned piglets fed diets with adequate or reduced LNAA levels.

Material and Methods: A total of 720 piglets with intact tails were assigned to one of 10 treatments in a 5×2 factorial design, comprising 5 levels of standardized ileal digestible (SID) Trp:Lys ratios (0.16, 0.20, 0.24, 0.28, and 0.32) and 2 levels of LNAA (100% or 85% of CVB requirements), with 12 replicate pens (6 piglets, balanced for sex) per treatment. Piglets were individually weighed and classified into body weight (BW) classes (light, medium, and heavy) at weaning. Performance data were collected over the whole period, and behavioural observations were conducted in weeks 3 and 5.

Results and Discussion: Feeding the LNAA 100% diet resulted in greater average daily gain (ADG) and gain-to-feed ratio (G:F) than the LNAA 85% diet across all BW classes ($P<0.01$). A Trp x BW class interaction was found for G:F ($P<0.05$). Within BW classes, dietary Trp affected G:F in heavy piglets, whereas no effect was detected in light or medium piglets. The response of G:F to dietary Trp in heavy piglets was nonlinear, with the highest G:F found at Trp 0.28, values being higher than for Trp 0.20 and 0.32. Neither Trp nor LNAA affected tail or ear biting ($P>0.05$), although both behaviours increased from week 3 to week 5. Aggressive behaviour decreased over time ($P<0.05$) and was lower in piglets fed Trp 0.24 and 0.28 than in those fed Trp 0.16 ($P<0.01$). Belly nosing was reduced in piglets fed Trp 0.20 and 0.24 compared with Trp 0.16 and 0.32 ($P<0.05$). Heavy piglets exhibited less ear biting and belly nosing than light and medium piglets ($P<0.05$).

In conclusion, Trp:Lys ratios of 0.24–0.28 improved G:F in heavily weaned piglets while reducing belly nosing and aggression, independent of LNAA supply. Adequate dietary LNAA also improved growth performance across all BW classes.

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Dietary fiber inclusion in the diet influences behavior and promotes satiety in group-housed gestating sows

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Introduction: During gestation, sow diets have been formulated primarily to meet nutritional requirements associated mainly with productive performance. In addition, diets for gestating sows have generally been characterized by low fiber content and high energy density (9). Another relevant aspect is restricted feed allowance (e.g., once daily), which has been adopted to prevent obesity and other health- related problems (2). In this context, physiological aspects such as satiety (3) and the proper functioning of the gastrointestinal tract are often neglected in commercially adopted nutritional and feeding programs. These practices expose animals to the recurrent risk of experiencing a state of hunger (2), thereby compromising animal welfare (5). The inclusion of fiber in gestation diets may increase postprandial satiety as a consequence of delayed gastric emptying, increased intestinal fill, and its influence on plasma metabolites and hormones (5,6). Therefore, the use of fiber sources in the diet is a strategy to mitigate the state of hunger in gestating sows. The aim of the present study was to evaluate the effects of including two levels of an insoluble fiber source in the diets of sows, as well as the inclusion of a soluble fiber source (soybean hulls), on behavior and satiety during gestation under group-housing conditions.

Material and Methods: A total of 112 gestating sows (217.8 ± 33.9 kg) were assigned to a randomized complete block design according to body weight and parity order (3.6 ± 1.2) and allocated to two batches with four dietary treatments (n = 28): 1) a control diet based on sorghum, corn, and soybean meal; 2) a diet including 2.0% of a commercial insoluble fiber (IF) source; 3) a diet including 4.0% of the same commercial IF source; and 4) a diet including 18% soybean hulls as a soluble fiber source (7% SF). The sows were housed in group-gestation pens with 14 females per pen and transferred to farrowing crates at 110 days of gestation. During gestation, sows were fed once daily (07:00 h) following a high-low-high feeding program. The amount of feed provided for each treatment was adjusted to equalize daily metabolizable energy and digestible lysine intake according to the genetic line recommendations. The sows were observed once weekly using the scan sampling method for behavioral assessment. Observations began 1 hour after the morning feeding, and each sow was evaluated at 8 time points throughout the day at 1- hour intervals. Instantaneous scan sampling was used to record the following behaviors: 1) inactive behaviors: lying and standing; 2) active behaviors: walking, drinking, and exploratory behavior; and 3) agonistic/negative behaviors: stereotypies and agonistic interactions. To assess satiety status, blood samples were collected on day 110 of gestation from 14 sows per treatment. Samples were collected early in the morning under fasting conditions, and subsequently at 2 and 4 hours after feeding, in order to evaluate serum GLP-1 concentrations.

Data were assessed for normality using the Shapiro–Wilk test (PROC UNIVARIATE, SAS Studio University Edition). For GLP-1, each sow was considered the experimental unit, and data were analyzed using the PROC GLIMMIX procedure with a mixed linear model including dietary treatment as a fixed effect and parity order, initial sow body weight, and pen as random effects. For behavioral variables, the pen was considered the experimental unit, and data were transformed using the natural logarithm of the ratio between the relative frequency of active or negative behaviors and inactive behaviors, with adjusted means back-transformed by exponentiation. Transformed data were analyzed using a mixed model including the fixed effects of group, treatment, hour, and treatment × hour interaction, with pen as a random effect (with hour nested within week and week nested within pen) and a first-order autoregressive correlation structure [AR(1)] applied to the residuals within pen. Stereotypic and agonistic behaviors (0/1) were analyzed using a generalized linear mixed model with binomial distribution and logit link function, including pen as a random effect. Body lesions were analyzed using a linear mixed model with group and treatment as fixed effects, pen as a random effect, and an AR(1) covariance structure for the residuals. Adjusted means were compared using Tukey's test, considering P

≤ 0.05 as significant and 0.05 < P ≤ 0.10 as indicating a tendency.

Results and Discussion: The use of different fiber types and sources in the diet influenced stereotypic behavior in gestating sows (P < 0.05; Table 1). The inclusion of SF (7%) reduced the occurrence of stereotypies compared with the control diet and the diet containing 4% IF (P < 0.05). Fiber-containing diets also tended to affect the ratio of active and negative behaviors relative to inactive behaviors (P ≤ 0.10). Sows fed the diet with 2% IF were more inactive, whereas those fed 4% IF showed greater activity compared with sows receiving 7% SF and 2% IF. In addition, there was a tendency for a lower occurrence of negative behaviors in sows fed 7% SF compared with the control and 4% IF treatments (P ≤ 0.10). GLP- 1 concentrations were influenced by sampling time (P = 0.01), and a treatment × time interaction was observed (P = 0.03), with a tendency for a treatment effect (P = 0.08). Under fasting conditions, animals receiving the 7% SF treatment showed higher GLP-1 concentrations than those fed 4% IF (P < 0.05). At 2 h postprandial, animals from the control group showed higher GLP-1 concentrations than those fed the diet containing 2% IF. The presence of stereotypies in gestating sows is strongly associated with negative emotional states, such as hunger, and with the failure to meet their behavioral and physiological needs under intensive production systems (7). Previous studies have reported beneficial effects of providing diets with lower nutrient density on feed intake patterns and, consequently, on the reduction of stereotypic behaviors associated with satiety (4). Furthermore, the results related to body lesion scores and plasma GLP-1 concentrations, together with the behavioral findings, suggest that the reduced signs of hunger such as lower feeding motivation, reduced physical activity, and fewer body lesions were likely associated with an increased state of satiety and improved welfare (8).

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The satiating potential of mice versus dry food and moist food in cats

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Introduction: Overconsumption of energy from foods underlies the development of overweight and obesity, which is unfortunately common in pet cat populations[1]. Reducing food intake by controlling appetite is therefore a way to combat feline obesity. The satiating potential of dry extruded and moist retorted foods is still understudied. It can be hypothesised that the potential is less than that of prey animals like mice typically consumed by cats in nature. For example, relative to dry foods, properties of prey animals favourable for maintaining satiety include low energy density (high volume), high contribution of protein to total metabolizable energy and intact complex physical structures (tissues). We therefore tested this hypothesis and compared the satiating potential of mice to that of a commercial dry food and a moist food using a model of operant conditioning to quantify food motivation.

Material and Methods: Before the start of the experiment, 20 cats were trained for 30 days to operate the conditioning apparatus, and afterwards were tested for mice consumption for 7 days. From these cats, 12 were selected for the experiment. These cats were fed dry food (Perferct Fit™ Indoor 1+ with chicken, Mars Petcare, Verden, Germany), moist food (Whiskas 1+ Natvoer Classic Selectie in Saus, Mars Petcare, Verden, Germany), and mice (Carus rodent facility of Wageningen University and Research, Wageningen, The Netherlands) according to a Latin square design. Cats were blocked by sex and randomly assigned to one of three groups starting with either dry food, moist food or mice. Each period lasted 7 days with testing for food motivation on days 4 and 7. As maximal four cats can be tested per day, three subgroups of cats were formed with subgroup 2 started one day later than subgroup 1 and subgroup 3 started one day later than subgroup 2. Food motivation was tested ~5 h after the morning meal of the experimental food (8:00), which was given at 50% of their estimated daily metabolizable energy requirements. Frozen mice were thawed in a fridge overnight and then put in a plastic bag to be warmed up in water. Warm mice were cut in four pieces (or smaller for some cats) and weighed amounts were fed to the cats. Cats were fed at 15:00 the dry food to cover the remaining 50% of their energy requirements. Weights of leftovers of each meal and potential regurgitated food were recorded. Operant test procedures were in line with previous work[2]. Work effort to obtain a single kibble (Perferct Fit™ Indoor 1+ with chicken) as a reward was increased in a progressive ratio (PR) based on experience and eagerness with pressing the lever[3], i.e. cats had a PR of +1 (n=8), +2 (n=3) or +3 (n=1). The total number of rewards received, the total number of lever presses, and the time duration of the tests were measured. Body weight of cats was measured before, during and at the end of the experiment. The linear mixed models for number of rewards, number of presses, and test times included diet, test period, test number, and morning food intake percentage as fixed factors, and cat ID as a random factor. Assumptions for normality and linearity of the residuals were tested and met using histograms and Shapiro-Wilk tests for normality, and residuals versus fitted plots for linearity.

Results and Discussion: During the experiment, average morning food intake of dry food was 98% (ranging from 0 to 100%), of moist food was 100% (ranging from 93 to 100%), and of mice was 79% (ranging from 0 to 100%). Out of the twelve cats, three were not able to complete the mouse diet period and had to be switched to dry food after 3 d and one cat was switched to dry food on day 7. All cats except one lost some weight during the experiment. On average the cats lost 2.5% in body weight (ranging from -0.3% to 5.4%). These changes in weight were not significant (P=0.86). Motivation for food was affected by food type (P=0.003). When cats were fed mice in the morning, fewer rewards were obtained (average 18 rewards, range 1-26) than when cats were fed dry food (22, 8-32) (P = 0.004). Cats that were fed moist food obtained similar numbers rewards (20, 3-36) as cats fed dry food (P=0.26) and cats fed mice (P=0.15). Motivation to perform the test was also affected by food type (P<0.001). When cats were fed mice in the morning, test times were shorter (9.4 min, 2.5-15 min) than when cats were fed dry food (13.1 min, 8.5-15 min) (P<0.001). Cats fed moist food also spend less time performing the tests (11.2, 3.5-15 min) than cats fed dry food (P=0.04), while spending similar times as cats fed mice (P=0.11). Test period, test number, and morning food intake did not affect food motivation (test period P=0.47, test number P=0.10, intake percentage P=0.41) or motivation to perform (test number P=0.13, test period P=0.23, intake percentage P=0.32). Differences among diets were stable over time, rather than varying across test periods (P>0.05). This study demonstrates that mice are more satiating than dry food, while having a similar satiating potential as moist food. Compared to dry food and moist food, mice were highest protein, intermediate in energy density or volume and were composed out of intact structures. Fibers types and amounts differed as well. When converted to g/MJ ME, the protein contents of the experimental diets were estimated to be 24.0 for dry food, 24.2 for moist food, and 28.7 for mice, which falls within the protein range tested in previous work, and would suggest a difference in satiating potential based on protein content[2]. The difference physical structure might also contribute to the differences in satiating potential of foods[2], although mice and moist food had similar potential. The latter might relate to a difference in energy density[4,5] with cats receiving on average ~75 g more moist food than mice. The relative contributions of each of the factors to the satiating potential of foods warrant further study. In conclusion, the tested dry food had a lower satiating potential than mice and the moist food had intermediate and similar potential as mice.

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In vitro mineral accessibility in commercial vegetarian and vegan extruded dog foods

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Introduction: The increasing use of vegetarian and vegan dog foods raises concerns about mineral adequacy, because plant-based ingredients may contain anti-nutritional factors such as phytate and fibre that can reduce mineral accessibility [1,2]. In addition, the processing history of plant protein ingredients may influence nutrient release from the food matrix [3]. We hypothesised that commercial extruded dog foods containing plant-based protein sources, especially entire plant ingredients, would show lower mineral accessibility than conventional animal-based foods.

Material and Methods: Twenty-two commercial dry extruded foods for adult dogs were evaluated, including seven foods with animal-based protein sources (AB), eight with entire plant-based protein sources (PB_e), and seven with plant protein concentrates or isolates (PB_{ci}). Samples were analysed for dry matter, nitrogen, minerals, and phytate. A two-step in vitro digestion procedure based on Hervera et al. [4] was applied in triplicate. Mineral contents (Ca, Mg, Zn, Fe, Cu, S, Se) were analysed with ICP-OES in the foods. Mineral accessibility, dry matter digestibility, and nitrogen digestibility were calculated. Mineral contents were compared against FEDIAF guidelines. Effects of food type were analysed using one-way ANOVA; for variables without normal distribution, the Kruskal-Wallis test was applied Post hoc analysis was done using the Turkey-HSD test at a significance level of $p < 0.05$ to detect significant differences among the three food types.

Results and Discussion: Only eight of the 22 commercial dog foods met all minimum recommended mineral concentrations. Several plant-based foods, particularly those containing entire plant ingredients (PB_e), were below recommendations for Ca, Zn, Cu, and Se. AB foods displayed lower Cu accessibility compared with the PB_{ci} foods ($p < 0.05$). Calcium accessibility was lower in PB_e foods compared with animal-based foods ($p < 0.01$), highlighting a potential nutritional concern. Dry matter digestibility was also lower in PB_e foods ($p < 0.05$), which may reflect the higher fibre content and structural complexity of plant-based ingredients.

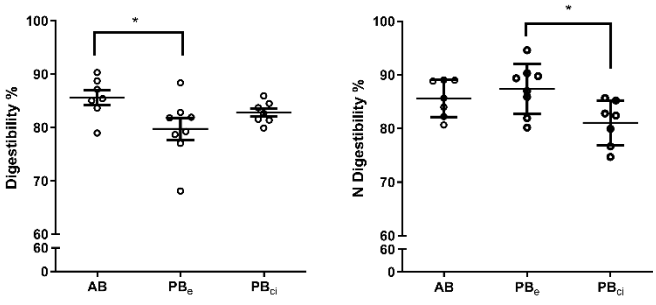

Figure 1. *In vitro* dry matter and nitrogen digestibility (%) of commercial extruded dog foods containing animal-based or plant-based protein sources. The middle lines show the mean; error bars represent the standard error. * $p < 0.05$.

In conclusion, the notably low accessibility of Ca in dog foods containing intact plant-based proteins, along with several foods showing Ca concentrations below FEDIAF recommendations, underscores the need for further monitoring of Ca status in animals fed these foods. Other observed differences in mineral accessibility between food groups could not be attributed to expected antagonistic factors, such as phytate and low protein digestibility.

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Table 1. In vitro mineral accessibility (%) of commercial extruded dog foods containing animal-based or plant-based protein sources.

	Zn	Mn	Fe	Cu	K	S	Se	Ca	Mg
AB	91.5±4.2	93.5±2.8	67.2±10.8	80.5±8.2	97.5±0.5	60.2±7.0	64.2±6.7	95.6 (94.6–96.2)	95.8 (94.9–96.5)
PB_e	88.5±5.3	89.8±4.2	59.5±6.5	87.2±6.3	96.0±1.0	49.3±25.7	69.2±11.8	91.2 (87.8–91.5)	91.9 (89.2–93.7)
PB_{ci}	88.1±5.0	89.7±4.7	57.5±9.3	90.1±3.9	96.2±0.7	61.4±13.6	72.8±9.0	92.4 (90.9–94.5)	93.4 (92.2–94.4)

Values are presented as mean ± SD for Zn, Mn, Fe, Cu, K, S, and Se, and as median (IQR) for Ca and Mg.

The effect of additional calcium and insoluble fibre on performance and calcium metabolism in weaned piglets

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Introduction: The post-weaning period is critical for pig development e.g. growth, skeletal integrity, and metabolic regulation. Vitamin D plays a key role in calcium homeostasis, yet excessive dietary calcium may impair its metabolism. Straw, as an indigestible fibre, may interfere with lipid absorption, thereby potentially affecting the absorption of vitamin D. We hypothesised that interactions between dietary calcium (limestone) and insoluble fibre (straw) would impact growth performance and modulate calcium utilisation and vitamin D status.

Material and Methods: A total of 144 weaned piglets (28 days old) were assigned to four dietary treatments in a randomized complete block design: control (L−S−), limestone (L+S−; 0.2% limestone), straw (L−S+; 5% straw), and limestone + straw (L+S+). The 38-day experiment included six replicate pens per treatment with six piglets per pen. Growth performance was recorded throughout the trial. Blood samples were collected on days 38 to determine vitamin D metabolites using UHPLC–MS/MS, and fecal samples were analyzed to evaluate mineral solubility.

Results and Discussion: Limestone or straw alone had limited effects on growth performance in weaned piglets. However, significant limestone × straw interactions were observed for body weight at day 38 (P = 0.05), as well as for ADG (P = 0.02) and ADFI (P = 0.01) during the first two weeks post-weaning. The interaction also affected ADG over the entire experimental period (P = 0.05), indicating that the combination of dietary calcium and insoluble fibre can modulate early growth more than either factor alone. Limestone supplementation increased FCR (P = 0.02) and significantly elevated fecal calcium and acid-soluble calcium concentrations (P < 0.01), while phosphorus and magnesium remained unaffected, suggesting altered calcium handling without altering other minerals. Interestingly, straw—but not limestone—reduced circulating concentrations of D3 (P = 0.05), 25OHD3 (P = 0.04), and 3-epi-25OHD3 (P = 0.07), indicating that dietary insoluble fibre may negatively influence vitamin D metabolism, potentially through effects on intestinal absorption or microbial fermentation. These findings highlight the importance of post-weaning diet formulation strategies balancing fibre and calcium to preserve vitamin D status.

Species-specific effects of dietary calcium on psittacine blood biochemistry

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Introduction: In captive Psittaciformes, attention has historically focused on the consequences of calcium deficiency, largely because parrots have traditionally been maintained on seed-based diets that are typically low in calcium. However, excessive dietary calcium has also been linked to adverse outcomes, most notably renal pathology, which in severe cases may lead to mortality (Phalen, 2009). Despite the fundamental importance of calcium metabolism in avian physiology, knowledge of species-specific tolerance to elevated dietary calcium levels in parrots is largely lacking. This comparative study evaluated interspecific variation in calcium metabolism and tolerance in three phylogenetically and biogeographically distinct parrot species: cockatiels (*Nymphicus hollandicus*, Australia), green-cheeked conures (*Pyrrhura molinae*, South America), and peach-faced lovebirds (*Agapornis roseicollis*, Africa).

Material and Methods: Fifty-four young female parrots (18 per species; 4–8 months of age) were weighed 2 weeks after arrival and randomly assigned to 3 dietary treatment groups. Birds were housed in 9 aviaries (3 replicates per treatment), each containing 2 birds of each species. After 1 month on a common seed diet, baseline blood samples were collected. Birds were then gradually transitioned to *ad libitum* extruded pellet diets differing in calcium content - low (0.7% Ca), medium (1.0% Ca), or high (1.4% Ca) - with a similar calcium-phosphorus ratio (Ca:P ≈ 1.6). After a 4-week transition period and 8 weeks on pellets only, a second blood sample was collected. Blood parameters (phosphate, uric acid, albumin, total calcium, and adjusted calcium) were measured in both samples. In addition, after 1 month on pellets only, birds were housed individually for 5 days to measure daily food and water intake.

Results and Discussion: Total calcium increased significantly in lovebirds ($p = 0.005$) and cockatiels ($p < 0.001$) fed the high-calcium diet, as did adjusted calcium levels in both species ($p < 0.01$). No significant changes were observed in green-cheeked conures. Albumin increased significantly only in cockatiels ($p = 0.013$). Dry matter intake per unit body weight was consistently lower in cockatiels than in the other species ($p < 0.01$), regardless of diet. Water intake, uric acid, and phosphate did not differ significantly among diets or species ($p > 0.05$). These findings demonstrate species-specific alterations in calcium metabolism in response to dietary calcium level, while unchanged water intake suggests that overt renal dysfunction was not evident within this phase of the study.

References: Phalen DN. 2009. Proc. AAVAC Annual Conf., Adelaide, pp. 239-242.