WAGENINGEN
UNIVERSITY & RESEARCH

PROGRAM BOOK

THE 45TH ANIMAL NUTRITION RESEARCH FORUM

Bridging expertise: advances in animal nutrition through interdisciplinary research

April 20th 2023

Wageningen, The Netherlands

WELCOME

Dear colleagues,

It is a great pleasure to welcome you in Wageningen, The Netherlands, to attend the 45th Animal Nutrition Research Forum on 20th of April 2023. This scientific forum was originally initiated by Wageningen University and Research, Utrecht University, KU Leuven, Antwerp University, and Ghent University to gather young researchers in the field of animal nutrition and provide them the opportunity to share their expertise.

This forum is the result of the hard work and dedication of many people, and we are grateful for everyone's contributions. Our theme this year, Bridging Expertise: Advances in Animal Nutrition through Interdisciplinary Research, reflects the importance of collaboration and interdisciplinary approaches in advancing our understanding of animal nutrition and its impact on the other animal aspects. Therefore, we have an excellent lineup of presenters who will be sharing their insights with us during the forum.

We believe that this event will be a valuable opportunity for networking, learning, and sharing ideas with professionals from different institutions and universities. We hope that you will take full advantage of the opportunities presented by this forum, and that you will leave with new knowledge, new connections, and a renewed sense of enthusiasm for your work.

Thank you for being a part of this event, and we look forward to an exciting and productive forum together.

Sincerely,

ANR2023 Organizing Committee

On behalf of Animal Nutrition Group, Wageningen University & Research,

Thomas Bastiaansen
Margreet Edens
Mochammad Fahmi Habibi
Ilaria Minussi
Hanlu Zhang

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ORGANIZING COMMITTEE

Mochammad Fahmi Habibi

Thomas Bastiaansen

Margreet Edens

Ilaria Minussi

Hanlu Zhang

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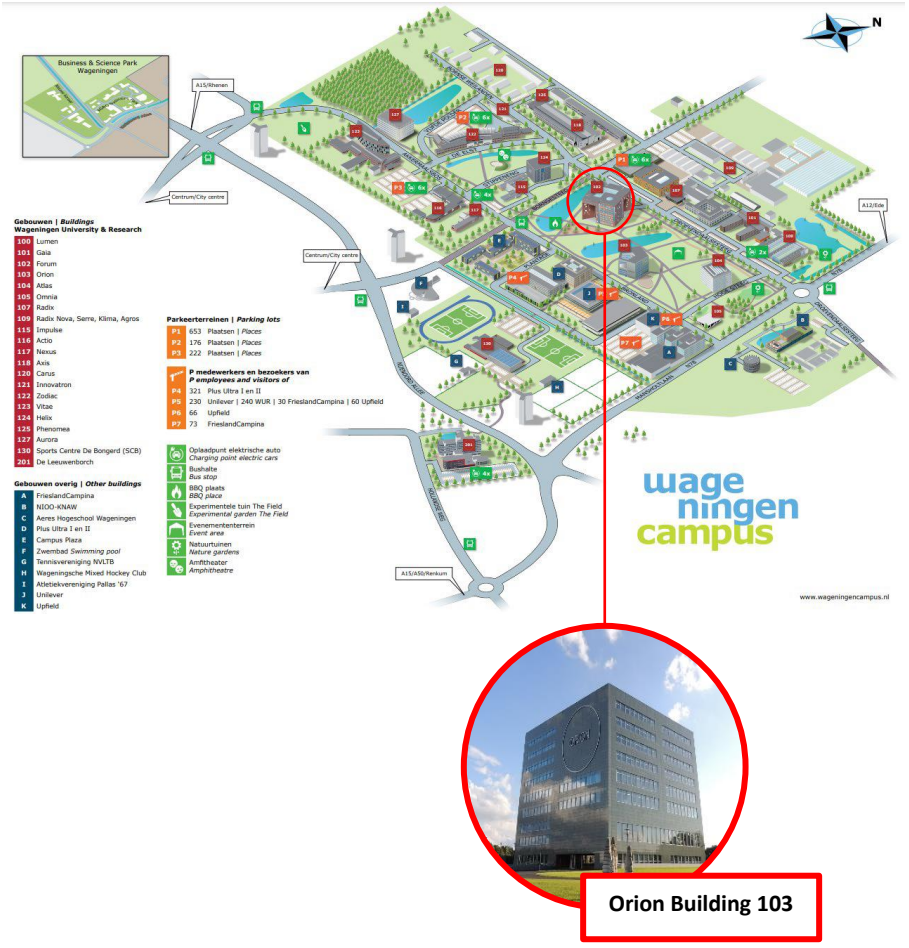

Orion Building 103

Conference room: **Room C2050** (accessible from 1st floor)

Drink and lunch spot : **The Spot** (ground floor)

GENERAL SCHEDULE

Time	Activity	Location
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9.00-9.20h	Opening	Room C2050
9.20-10.20h	Session 1 - Oral presentation and poster pitches	Room C2050
10.20-10.50h	Coffee break and posters	Pantry room C2050
10.50-11.50h	Session 2 - Oral presentation and poster pitches	Room C2050
11.50-12.50h	Lunch break and posters	The Spot, Orion
12.50-14.30h	Session 3 - Oral presentation	Room C2050
14.30-15.00h	Coffee break and posters	Pantry room C2050
15.00-15.45h	Session 4 - Oral presentation	Room C2050
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16.00-18.00h	Drinks	The Spot, Orion

DETAILED SCHEDULE

Location: Wageningen University Campus
ORION, Building 103, Bronland 1, 6708 WH Wageningen

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	O2: The impact of feeding hay on pre-weaning piglets gut development: implications for optimizing creep feeding strategy	Renjie Yao
	O3: Milk yield and quality parameters with different levels of roughage use in metropolitan dairy farming systems	Md Shahin Alam
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	O5: Effect of extrusion and phytase on the digestibility of plant-based dog food	Yunhan Zhang
	O6: Improving ruminant digestibility of various rice straw cultivars by white-rot fungi	Hai Tran
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	O8: Effect of bedding type and feeding level on performance and metabolic responses in ducks (Anas platyrhynchos domesticus)	Awot Mebratu
	O9: Felid Feeding in Zoos: Is body mass the key?	Mengmeng Sun
	O10: Feed intake and milk production of dairy cows fed with a ration with ensiled tall fescue compared to ensiled perennial ryegrass	Maarten Cromheeke
	O11: Time to first meal and its impact on performance in conventional and slow growing broiler breeds	Niamh Cleiren
	O12: Lifelong effects of different creep feed administration methods on production performance of pigs	Katrijn Hooyberghs
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ABSTRACTS

O1

Dietary sodium butyrate improves female broiler breeder performance and offspring immune function by enhancing maternal intestinal barrier and microbiota

Chuanpi Xiao[1]*, Zhigang Song[2]

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This study aimed to investigate the effects of dietary sodium butyrate (SB) supplementation on the reproductive performance of female broiler breeders under intensive rearing conditions and to analyze antioxidant capacity, immune function, and intestinal barrier function of the female breeders and their offspring. A total of 96,000 40-week-old Ross308 female broiler breeders were divided into the control (CON) and SB groups, each with six replicates of 8,000 birds. Each house with similar production parameters was considered a replicate. The experiment lasted for 20 weeks, whereupon sampling took place. Results showed that SB improved the egg production performance, egg quality of broiler breeders, and hatchability ($P < 0.05$). Maternal supplementation with SB substantially increased serum immunoglobulin A levels in broiler breeders and offspring and offspring immunoglobulin G ($P < 0.05$). The levels of interleukin-1β and interleukin-4 in the offspring were downregulated, while the superoxide dismutase in the offspring and the eggs increased ($P < 0.05$). SB altered the serum biochemical parameters in breeders and offspring, as evidenced by the reduction in triglycerides, total cholesterol, and high- and low-density lipoproteins ($P < 0.05$). SB improved the intestinal morphology of broiler breeders and offspring by decreasing the jejunal crypt depth ($P = 0.04$) and increasing villus height in offspring ($P = 0.03$). Maternal jejunal and ileal intestinal barrier-related genes were also shown to be significantly affected by SB. Furthermore, SB altered the microbial diversity in maternal cecal contents, thus increasing the abundance of Lachnospiraceae ($P = 0.004$) and Ruminococcaceae ($P = 0.03$). Dietary SB enhanced the reproductive performance and egg quality of broiler breeders and improved the antioxidant capacity and immune parameters of broiler breeders and offspring, with the benefits potentially arising from the regulation of the maternal intestinal barrier and gut microbiota by SB.

O2

The impact of feeding hay on pre-weaning piglets gut development: implications for optimizing creep feeding strategy

Renjie Yao[1], An Cools[1], Hubèrt M.J van Hees[3], Marijke Aluwé[4], Dominiek Maes[2], and Geert P.J Janssens[1]

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[2]Department of Internal Medicine, Reproduction and Population Medicine, Merelbeke, Belgium
[3]Trouw Nutrition Research & Development, Amersfoort, The Netherlands
[4]Flanders Research Institute for Agriculture, Fisheries and Food (ILVO), Melle, Belgium

The effect of dietary fibre on pig production has been extensively evaluated so far. Insight of wild boar gastric content at an earlier age, the study aimed to examine the benefits of feeding grass hay to pre-weaning piglets apart from commercial creep feed. 15 sows were divided over two groups based on balanced parities. The control group (CON, n=7) only received commercial creep feed, while the treatment piglets (GH) were provided with cut grass hay until weaning (28d) in addition to the same creep feed. At weaning, one piglet per sow with a median weight was euthanized. The morphometry of gastrointestinal tract was examined, and the content in mid-colon were collected for microbiota analysis through 16S rRNA amplicon sequencing. The DADA 2 was used to process the data into ASVs according to pipeline tutorial. The microbiome data analysis was performed by vegan package in R and phyloseq. The results of morphology were evaluated through GLM. Differences were considered if $p<0.05$. The weight of empty small and large intestine was greater in GH (285 vs 224g, 91 vs 77g respectively, $p<0.05$). In the results of microbiome community, at phylum level, the Firmicutes and Bacteroidota were the most prevalent (over 95%). Although different clustering of the microbiome community did not emerge in principal coordinate analysis, there was a trend that Lactobacillus proportions at genus level were greater in GH than in CON (19.6 vs 8.0%, $p=0.08$). Lactobacillus are known for producing energy source for the cell line the colon, and maintaining the integrity of gut barrier, which may contribute to preventing the growth-hurdle and obtain stronger gut. Due to above, providing grass hay to suckling piglets stimulated maturation of digestive system and seems a promising strategy for health post-weaning.

O3

Milk yield and quality parameters with different levels of roughage use in metropolitan dairy farming systems

Md Shahin Alam[1], Regina Roessler[1], Anjumoni Mech[2], Nira Manik Soren[2], Eva Schlecht[1]*

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Milk yield and quality, especially the fatty acid (FA) profile, are influenced by the ration of dairy cows. Particularly metropolitan dairy farmers face severe shortage of cattle feed due to huge scarcity of arable land for forage production, in particular roughages. To explore the effect of the dietary roughage share on milk FA profile, 39 farmers in the Greater Bengaluru metropolitan area were visited repeatedly. Individual cow data and required samples were collected on breed, body weight (BW), milk yield and quality, as well as diet composition in winter 2021 (n=74, BW=400 ± 63 kg), monsoon 2021 (n=61, BW=399 ± 63 kg), and summer season 2022 (n=104, BW=399 ± 72 kg). Farmers fed diverse roughages at an average daily dry matter (DM) amount of 6.79 ± 1.56 kg/cow; this included finger millet straw, food leftovers, and grasses from Napier, maize and lakeshore. In addition, concentrate was offered at 2.98 ± 1.49 kg DM/cow, namely wheat bran, groundnut cake, and feed pellets. On average, 0.92 ± 0.47 kg milk were produced per kg of consumed DM. Cows with a roughage share of < 70% in their diet (n=117) had higher ($P < 0.01$) milk yield (14%) than cows with > 70% roughage share (n=122), but there were no differences in milk fat content. Yet, at > 70% roughage share, cow milk contained 10% more unsaturated FA, 6% less saturated FA ($P < 0.01$) as well as 6% ($P < 0.05$) and 21% more oleic and linoleic acid, ($P < 0.01$). Furthermore, with < 70% roughage, higher concentrations of methyl myristate (8%) and methyl palmitate (23%) were determined ($P < 0.01$). In conclusion, the FA profile is healthier when cows are fed a roughage-rich diet, which is a major challenge for the investigated metropolitan dairying.

O4

Nutrient digestibility and digesta mean retention time in laying hens of two different breeds fed with fine vs coarse oat hulls

Mochammad Fahmi Habibi[1,2], Rene Kwakkel[1], Sonja de Vries[1]

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To optimally acknowledge the feeding value of dietary fibre, effects of dietary fibre on digestive processes need to be investigated. Previous studies reported that chicken breeds selected for certain production traits or digestion efficiency differ in their response to fibrous diets. An experiment was performed with 2x2 factorial arrangement with two 29-wk old laying hen breeds (DeKalb White (DW) and Bovans Black (BB)) and two oat hull (OH) structures (fine and coarse) to investigate their effects and interaction on nutrient digestibility and digesta mean retention time (MRT). The diets were formulated to contain 15g/kg OH as the only fibre source, TiO2 (3g/kg) as a marker for solid digesta, and Co-EDTA (2g/kg) for liquid digesta. At 33 wk of age, birds were euthanized and digesta from crop, proventriculus+gizzard, duodenum+jejunum, ileum, and ceca+colon were analysed for marker and nutrient concentrations. Apparent ileal digestibility (AID) of nitrogen was greater (+3.8%-units, P<0.01) in BB vs DW hens, whereas AID of starch was only higher in BB when the fine OH diet was fed (breed×diet, P=0.04). Contrastingly, apparent total tract digestibility (ATTD) of dry matter (-2.5%-units, P<0.01), nitrogen (-8.1%-units, P<0.01), fat (-4.2%-units, P<0.01), and NSP (-0.5%-units, P=0.03) were lower in BB vs DW hens. A higher AID for nitrogen coincided with longer retention of solid digesta in proventriculus+gizzard (+25min, P<0.01) and ceca+colon (+11min, P<0.01), and of liquid digesta in small intestine (+10 to 12min, P<0.01) in BB vs DW hens. Moreover, OH particle size had only a tendency for greater ATTD of fat (+2%-units, P=0.09) and a reduced retention of solid digesta in the ceca+colon (-8min, P<0.05) for coarse vs fine OH. Overall, our study indicates marginal differences in responses to OH particle sizes between breeds. Nutrient digestion and MRT, however, differed between breeds, regardless of diet.

O5

Effect of extrusion and phytase on the digestibility of plant-based dog food

Yunhan Zhang[1], Geert P.J Janssens[1],Donna Vanhauteghem[1], Marielle K.V Zelst[2], Mingyang Wang[2], Leon Marchal[2], Guido Bosch[2]

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Replacing animal-based protein sources with plant-based protein sources has been proposed to improve the sustainability of pet food. Besides, extrusion technology and phytase addition, as common processing methods, may impact the bioavailability of nutrients. This study aimed to determine the effect of extrusion and phytase on the digestibility of various plant-based pet foods using an *in vitro* digestion assay. Six different plant protein sources (pea, faba bean, lentils, kidney bean, chickpea, sunflower, rapeseed) were included at a rate of 50% in formulation and extruded using a twin-screw extruder. The protein, starch and fat levels were standardized by changing inclusion of corn, corn gluten meal and poultry fat. All foods need to be grinded with a mesh size of 1 mm, 0.5 g of each sample with 2 replicates were weight accurately to experience *in vitro* digestion simulation, following a modified procedure. The phytase was added during the simulation of stomach stage at 20000 FTU/kg food. The weight of each sample, and residue on dry matter basis was recorded to calculate digestibility on dry matter basis. Data were statistically analyzed by repeated measures ANOVA with Extrusion, Phytase addition and the interaction as fixed effects of the model. Extrusion increased DM digestibility by 7.81% ($p<0.001$), while the phytase did not change digestibility. The improvement on digestibility may be due to anti-nutritional factors that are inactivated by extrusion processing. The digestibility of different protein sources without phytase is between 65.4% and 87.9%, lentils showed the best potential at 87.9%, while rapeseed was only 65.4% digestible. Besides, pea, faba bean, chickpea also showed great potential on digestibility over 80%. More detailed analyses of nutrients (e.g., minerals) are pending and will provide more insight in the nutritional properties of these plant protein sources as well as the effect of phytase.

O6

Improving ruminant digestibility of various rice straw cultivars by white-rot fungi

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The breakdown of the lignin polymer is vital for efficiently using rice straw (RS) as animal feed. Much evidence has been reported on the lignin degradation efficacy of white-rot fungi (WRF), such as Ceriporiopsis subvermispora (CS) and Lentinula edodes (LE). The effectiveness of WRF is affected by several factors, including fungal strains, substrate and temperature. The effectiveness of treating RS by WRF is variable, making identification of the best fungal strain for a specific substrate essential for practical application. This research aimed to determine the effect of different RS cultivars on fungal growth and lignocellulose degradation by WRF. First, the growth rate of CS on 12 RS cultivars was investigated. Next, the efficacy of degrading lignocellulose of different RS cultivars by CS and LE was investigated. The growth rate of CS on different RS cultivars varied significantly ($P<0.05$). The growth rate was highest (5.1mm/d) on SV181 and OM5451 cultivars, which was 6.3 times the value of OM6976 and BT7 (~0.8mm/d). After seven weeks, CS degraded 63 to 74% of lignin in the cultivars where CS grew the fastest. Only 20 to 27% of total lignin was degraded when the fungi grew poorly. In contrast, LE caused significant lignin degradation in all straw cultivars, with 65 to 82% lost after seven weeks. Except for the SV181, the same trend was found for hemicellulose degradation by CS, with 50 and 30% losses for the fast and poor-growing groups, respectively. The LE degraded more hemicellulose than CS, with 55 and 38% losses in the two previous groups. There was no interaction between incubation time and straw cultivars on the absolute amount of cellulose ($P=0.197$). In conclusion, LE shows the most potential for treating RS for a practical application.

O7

Latency to feed access in broiler chicks: Effects on first feed intake, yolk absorption, blood glucose levels and body temperature

Rutger Smets[1], Niamh Cleiren[1], Evelyne Delezie[2], Marta Lourenço[2], Frank Tuyttens[2,3], Luc Duchateau[4], Tomas Norton[5], Gunther Antonissen[1]

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Good health and welfare are of major importance for the resilience and performance of broilers. From literature, it is known that time to first feed access can influence broiler development, however, it is not well documented when chicks start eating after hatching. In conventional poultry production, newly hatched chicks don't have access to feed until they arrive at the farm. Depending on the width of the hatching window (up to 24h), other hatchery procedures and transport the latency between hatching and the first meal can be up to 48h. To know when chicks start eating after hatching and how it is linked to blood glucose and yolk sac utilization, a trial was performed using Ross 308 and Hubbard Redbro broilers hatched on-farm. Therefore 960 birds per breed were hatched and randomly distributed over three groups: T0 with immediate access to feed, T1 with access to feed 24h after hatching and T2 with access to feed 48h after hatching. Water was available to all three groups immediately after hatching. Broilers were raised with a 3-phase feeding system and according to conventional practices. Each breed was raised in a different compartment to meet the required environmental. Crop filling, blood glucose levels and relative egg yolk weight were monitored throughout the trial. Preliminary data analysis (ANOVA) showed an effect of feed deprivation time on relative egg yolk weight only for the Hubbard broilers, where 24h after hatching, T2 broilers showed the lightest and T1 broilers the heaviest egg yolk ($P<0.054$), whereas 48h after hatching, T2 broilers had the heaviest and T0 broilers the lightest egg yolk ($P<0.022$). Statistical analyses for other variables is still ongoing. These preliminary results suggest a greater effect of post-hatch feed deprivation in Hubbard vs Ross broilers.

O8

Effect of bedding type and feeding level on performance and metabolic responses in ducks (*Anas platyrhynchos domesticus*)

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The present study evaluated the effect of bedding type and feeding level on performance, blood metabolites, and body nutrient composition of ducks. A total of 240 day-old ducklings were randomly divided over 24 floor-pens. To evaluate the effect of bedding type on feeding, pens were floor covered with either straw or wood shavings, and each group contained six sub-groups with ad lib (100%) and 95, 90, 85, 80, and 75% feed offers of the ad lib with a commercially available duck starter and grower pelleted-feeds. The experiment lasted for 42 days and ducks were fed on pen basis. Performance data were collected and ducks were sampled for blood, breast muscle, caecal contents. A mixed model with feeding level and bedding type was applied to the data. As expected, weight gain varied with feedinglevel ($P<0.05$) while only the 75% feedinglevel showed lower feed conversion ratio ($P<0.05$). Ducks raised on straw bedding showed higher weight gain and lower feed conversion ratio than those on wood shavings ($P<0.05$) with no effect on feed intake ($P>0.05$). Straw bedding had increased the ratio of acylcarnitines to free carnitine, in the duck blood indicating elevated metabolic rate ($P<0.05$) and yielded a better supply of glucogenic substrate to the citric acid cycle (propionyl-:acetylcarnitine; $P<0.05$). Moreover, straw bedding have also yielded increased breast muscle concentrations of several saturated and monounsaturated fatty acids compared with wood shavings ($P<0.05$). Caecal microbial evaluations revealed dominating Bacteroides and Firmicutes bacterial groups in ducks with straw bedding with Shannon and Simpson diversity indexes of 3.6 and 16.8, respectively. In conclusion, bedding type and feedinglevel both affect the performance of ducks that are associated with changes in the caecal microbiome and host metabolism.

O9

Felid Feeding in Zoos: Is body mass the key?

Mengmeng Sun[1]*, Annelies De Cuyper[1], Quiévy Alicia[2], Geert Paul Jules Janssens[1]

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Felid species range in their body masses from about 1 kg to about 300 kg. The body mass of mammalian carnivores is associated with natural hunting and feeding behavior (De Cuyper et al., 2018). The size of a carnivore can determine the selection of a particular prey size and the kill frequency. Large carnivores are most often described as 'selective' large prey feeders with low kill frequencies whereas small carnivores as 'non-selective' small prey feeders with high kill frequencies. Due to the differences in intake of fibrous animal matter, this may have co-evolved with digestive physiology. We performed a study with zoo felids of different body mass fed the same diet and evaluated the difference in fecal microbiota and fermentation products (short-chain fatty acids (SCFA), branched-chain fatty acids (BCFA)). Large and small felids differed (mildly) in some but not all fecal parameters. In zoos, keepers prefer to feed large carnivores on a daily basis. The most popular foods are usually raw meat diets, meaning no bones, cartilage, organs, skin or connective tissue. Some big cats, such as lions, may not receive important satiety signals when their energy needs are met with daily feeding. A major function of satiation is to prevent overconsumption during individual meals, thereby averting deleterious consequences from incomplete digestion. More meat protein may escape enzymatic digestion and excessive fermentation in the hindgut leads to higher concentrations of fecal SCFAs and their putrefactive compounds (indole, phenol, and p-cresol) (Depauw et al., 2013). When formulating diets for zoo felids, not only animal body mass should be considered, but also the natural hunting behavior because microbiota and fermentation may not be determined by felid body mass per se, rather by the ecological niche associated with a felid's body mass.

O10

Feed intake and milk production of dairy cows fed with a ration with ensiled tall fescue compared to ensiled perennial ryegrass

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Voluntary intake and digestibility of ensiled tall fescue (Festuca arundinacea) is proved to be lower compared to perennial ryegrass (Lolium perenne). However, new varieties of tall fescue have been bred to improve palatability and digestibility, but there is a paucity of results on intake and animal performance trials with these new varieties. This knowledge gap inhibits adoption in practice. At the same time, there is an increased interest in tall fescue as an alternative for perennial ryegrass in North-West European dairy production because of increased frequency of periods of summer drought that jeopardizes the production of high quality forage grass. In this feeding trial we investigated differences in feed intake and milk production (MY) of dairy cows fed with a ration with ensiled tall fescue compared to (diploid and tetraploid) perennial ryegrass. We measured intake and milk production of Holstein Friesian dairy cows fed with a forage ration of ensiled (1) tall fescue (Fa), (2) perennial ryegrass diploid (Lp2) or (3) perennial ryegrass tetraploid (Lp4). Cows were randomized in 6 groups of 5 cows which subsequently received each of the three treatments using a Latin-square design. Results showed a significantly lower total dry matter intake (DMI) of Fa (21,9kg) compared to the two Lp treatments (22,8kg Lp2 and 22,9kg Lp4) ($p < 0.05$). This difference was related to lower roughage DMI. Fat- and protein-corrected milk (FPCM) was significantly lower for Fa in comparison with Lp2 and Lp4 (respectively 32,8kg, 34,6kg and 35,5kg). There were no significant differences in milk composition (fat, protein and lactose) except for milk urea. These findings confirm lower DMI and FPCM of dairy cows fed a ration with ensiled tall fescue compared to ensiled perennial ryegrass. Similar trials will be set-up in the VLAIO KlimGras project.

O11

Time to first meal and its impact on performance in conventional and slow growing broiler breeds

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The first days of life are of critical importance in broiler production, as important developments have yet to take place during a short time span. In order to assess the impact of time to first meal on several performance, health and welfare parameters, a trial took place where Ross 308 and Hubbard Redbro broilers were hatched on-farm, in separate compartments to meet the environmental requirements of each breed. After hatching, they were randomly distributed over three treatment groups: T0 with immediate access to feed, T1 with access to feed 24h after hatching and T2 with access to feed 48h after hatching. Water was readily available for all treatment groups. Preliminary data analysis (ANOVA) showed significant differences in average daily feed intake (FI) and average daily growth (ADG) between the treatment groups, within each breed. For Hubbard broilers, FI decreased significantly with increasing delay of access to feed in the starter phase ($P < 0.01$), with T0, T1 and T2 broilers consuming 22.2g±1.2g, 20.2g±0.9g and 18.2g±1.3g respectively. A significant decrease was also present in the grower phase ($P = 0.042$), with Hubbard T0, T1 and T2 consuming 63.0g±4.9g, 58.6g±4.6g and 57.1g±3.6g respectively. For Ross, only T2 broilers (18.5g±1.1g) showed a significant decrease in FI compared to T0 broilers (22.0g±1.6g) during the starter phase ($P < 0.01$). Hubbard T2 broilers also showed a significantly lower ADG compared to T0 broilers during the starter phase ($P < 0.01$), with T2 broilers weighing 10.8g±1.5g and T0 broilers weighing 14.0g±0.6g, and grower phase ($P = 0.028$), with T2 broilers weighing 41.3g±2.6g and T0 broilers weighing 45.3g±3.7g. Based on these preliminary results, one can conclude that early access to feed has a significant impact on several performance parameters in both breeds, especially Hubbard, but these differences are no longer significant after the starter and grower phase.

O12

Lifelong effects of different creep feed administration methods on production performance of pigs

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Piglets experience stress at weaning which has an impact on their performance and health. Therefore, a creep feed (CF) is used to facilitate the transition and maintain performance. In this study, we hypothesized that using of the same bowl in the farrowing pen and post-weaning would increase performance and that the form of distribution (dry or liquid) would influence the magnitude of this effect. To test this hypothesis, two pig nursery trials were conducted to compare the two methods of CF administration and their effects on growth performance, feed intake, uniformity, feed conversion ratio (FCR) and carcass quality. In both trials, piglets received CF from an age of ten days until 1 week after weaning. The first trial included 120 crossbred piglets, half of which received weaning feed (WF) in a standard feeder, as well as CF in an additional bowl. The other half received CF and WF in the standard feeder. The second trial included 164 crossbred piglets. All piglets received WF in a standard feeder and dry or liquid CF in an additional bowl. Individual weights were recorded weekly until 9 weeks of age. Furthermore, daily weights were collected of the pigs of the first trial in feeding stations over a 125-day period. The additional bowl led to a significantly higher feed intake (or spillage) and FCR and a lower gain-to-feed during the first week after weaning. The liquid CF resulted in higher feed intake (or spillage) during the first week after weaning and a higher average daily gain was observed in the second week after weaning. Also, between 4 and 9 weeks, a higher FCR and a lower gain-to-feed ratio was found in the group with liquid CF. However, no long-term effects were found on performance nor on carcass quality.

O13

Fermentation of carbohydrate sources in different equine hindgut segments using a fully-automated *in vitro* gas production technique system

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Background: The horse is a hindgut fermenter and its well-being and gastro-intestinal health depend amongst others on the composition of the intestinal microbiota, where an imbalance can even be causal to disease (colic/laminitis). The cecum and colon are difficult to study in vivo because of their limited accessibility. An alternative method to in vivo experiments is the *in vitro* gas production technique (IVGPT) (Desrousseaux et al., 2012) that allows exploration of the impact of different carbohydrate sources by measuring the kinetics of gas production in equine cecum (CE), right ventral colon (RVC) and rectum (RE)1. Most equine IVGPT experiments have been performed with fecal inocula. Fecal samples are easily collected but it is questionable whether feces as inocula are representative of CE and RVC inocula in the IVGPT. Aim: Compare the use of RE inocula in the IVGPT with CE an RVC inocula. Methods: The IVGPT2 was used to measure gas production (GP) of hay (H), wheat bran (W), beet pulp flakes (S), cellulose (C), corn starch (M) and inulin (I) at 4h and 72h using CA, RVC and RE inocula. Experimental design: The experiment was a factorial design consisting of 6 substrates and 3 inocula. Data was analyzed by 2-way ANOVA; MIXED model procedure in SAS 9.4. Results: Ranking of inocula at 4h and 72h in ascending order for total VFA's was: REa<CAb<RVCb 3(range 14.6-34.4 mM) and REa<CAb<RVCc (range 77.9-98.8mM) respectively. For GP at 4h and 72h: CAa<REa<RVCb (range 44.6-78.4 ml gas/g OM) and CAa<RVCa<REb (range 262.8-284.4 ml gas/g OM). Conclusion: RE inocula is not representative for the extent and kinetics of microbial fermentation in CE and RVC inocula.

O14

Adapting *in vitro* incubation method of nitrogen digestion kinetics for different feed ingredient in pigs

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Digestion kinetics (digestion rate and extent), mostly measured *in vitro*, is critical to assess the nutritional value of diets for pig formulation practices. This study is aiming to adapt a Boisen-based *in vitro* digestion model to measure protein digestion kinetics and quantify the differences of protein digestion kinetics among different feed ingredients varying in protein content and in vivo protein digestibility. A similar amount of nitrogen originating from each of selected four feed ingredients (rapeseed meal, fishmeal, barley, peas) was subjected to a two-step enzymatic incubation. We simulated gastric conditions in two phases by lowering the pH during the pepsin incubation in the middle (90min at pH4, 90min at pH2), followed by small intestinal incubation for 240min with pancreatin at pH6.8. Total solubilized protein (TSP) was measured at several time points during incubation. The amount of solubilized protein during the first 90min varied among ingredients, ranging from 12 in rapeseed meal, 9 in fishmeal, 28 in barley and 26 in peas of total protein (%-units, $P < 0.05$). After changing pH, protein solubilized faster for fishmeal (0.59%-unit/min as linear increase) than other ingredients (0.28 for rapeseed meal, 0.15 for barley and 0.31 for peas of total protein, %-unit/min, $P < 0.01$). Following the stomach phase, TSP increased by 30%-units (rapeseed meal), 14%-units (fishmeal), 21%-units (barley) and 16%-units (peas) of total protein more ($P < 0.01$), reaching to 76% (rapeseed meal), 86% (fishmeal), 77% (barley) and 85% (peas) for total protein solubility at the end of incubation. The adapted method is sensitive for *in vitro* digestion kinetics measurement as it consistently varies among different feed ingredients with different protein types. Furthermore, the current results indicate that digestion kinetics are largely affected by pH changes in the stomach.

O15

Collecting and analysing pig mucosal scraping samples for mass spectrometry-based proteomics: a study towards the factors causing variation

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Proteomics is increasingly used in gut-related research in pigs (Sus scrofa), but detailed descriptions of sample collection methods and sample preparation are often lacking. We suspect several factors, such as inter- and intra-technician variability in the collection of samples, variability between pigs but also the day of analysis might affect proteomics results. To examine this variability, we conducted a reproducibility study in which mucosal samples were collected from 12 piglets fed two different diets (n=6 per diet). The piglets were weaned at four weeks of age and were fed ad libitum. At an age of seven weeks, the piglets were euthanised. A segment of 60 cm in the jejunum was identified and then divided in six equal pieces of 10 cm. Then, two persons collected scrapings, each from three segments, by scraping the surface of the intestinal wall twice using a microscope slide. The samples were then divided into two groups via controlled randomization, and these two groups were extracted and analysed on two separate days. Protein extraction was performed using S-Traps (ProtiFi), a sample processing technology using SDS, suspension trapping and trypsin. The so-generated peptide mixtures were analysed using liquid chromatography coupled to high resolution mass spectrometry (LC-HRMS). The quantification and identification of proteins was performed using Progenesis QI and Perseus was used for the interpretation of the results. Preliminary visual assessment of principal component analysis plots suggests differences between samples collected by different individuals, and a difference in within-person collected samples. Further statistical analysis is currently being performed.

P1

Can a saponin-aluminosilicate blend promote resilience to Eimeria infection?

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In broilers, coccidiosis leads to reduced growth and feed efficiency and is a predisposing factor of necrotic enteritis. Conventional strategies to control coccidiosis, chemoprophylaxis and vaccination, cannot fully prevent infection. Therefore, the use of feed additives to control *Eimeria* infection and improve gut health can bring added value to coccidiosis management strategies. A trial was performed to evaluate the effect of a blend of *Quillaja saponaria* extract (source of saponins) and an aluminosilicate in broilers challenged with coccidiosis by applying a used litter model. Due to the antiprotozoal activity of saponins and the toxin binding ability of aluminosilicates, it was hypothesised that the saponin-aluminosilicate blend would reduce oocyst shedding and improve performance of infected birds. In total, 1152 one-day-old Ross 708 male broilers were divided over four treatments, each with 12 replicates: a positive control reared on clean litter, with no additive or coccidiostats in the feed (PC); a negative control reared on used litter, with no additive or coccidiostats in the feed (NC); a NC with 60 ppm coccidiostat (salinomycin) added to the feed (NC+sal); a NC with the saponin-aluminosilicate (containing 30 ppm saponin extract) in the feed (NC+sap/al). Results were analyzed using ANOVA with the significance level set at 0.05. In case of significant effects, Tukey's multiple comparisons test was used to determine differences between treatment means. A significant effect was observed for final body weight (BW) at 42d (P=0.030) where NC had a lower BW than the NC+sal group. For feed conversion ratio (FCR) and the number of oocysts shed, trends were observed (P=0.053 and P=0.063, respectively). Notably, the number of oocysts shed between 22 and 24d was similar for NC+sal and NC+sap/al treatments.
The saponin-aluminosilicate blend could potentially be implemented as an alternative strategy to control coccidiosis challenge in broilers and further promote resilience to infection.

P2

Nutritional emulsifier as a cost-effective tool in sows lactation diet

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The start of sows' lactation is a challenging period, and optimal performance is required for a good piglets' health status. Nutritional emulsifiers are supporting emulsification and fat digestion in the gut. Study's objective was to evaluate the effect of a nutritional emulsifier on colostrum quality and performance of lactating sows and suckling piglets. Hypothesis was that, with the nutritional emulsifier, the energy in feed formulation can be reduced while not impacting the performance. Seventy-seven sows were followed from 5 days before farrowing until piglets' weaning, at 21 days. Sows were distributed, blocked by parity, in 2 groups, control and treated, with average parity of 2.85 for both. The control sows were fed a corn-based diet with 3450 kcal/kg metabolizable energy (ME), 20.8% crude protein (CP) and 6.5% crude fat (CF). The treated sows were fed a reformulated diet with reduction of ME by 50 kcal/kg, same CP and CF contents, and inclusion of a nutritional emulsifier based on glyceryl polyethyleneglycol ricinoleate. Sows and piglets were weighed at farrowing and weaning. Colostrum samples were collected within 12 hours after delivery and immunoglobulins G were analysed using Brix refractometry. Sows' daily feed intake (DFI) and piglets' weight gain were used to calculate feed conversion ratio (FCR). All parameters were analysed by SAS package and compared by a Tukey test at 5% significance. Finally, feed costs for control and treated sows were evaluated. The DFI, FCR, Brix value, sow and piglet weights for the treated sows were not different than the control ($P>0.05$). The lactation feed costs of the control and treated sows were 2.15 and 2.07 €/sow/day, respectively. In conclusion, feeding an energy reduced diet resulted in costs savings (160.16€ for 77 sows and 26 days), while maintaining colostrum quality and performance of sows and piglets, when supplemented with the nutritional emulsifier.

P3

Effects of technological treatment and particle size of grains on nutritional values and performance in dairy cows

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Technological treatment of grains may affect their nutritional values. The effects of average particle size (APS) and treatment of grains on degradation kinetics and nutritional values in rations for cows, possibly affecting rumen health and performance, were investigated. In a 6-wk performance trial, 72 mid-lactating cows were tested according to a randomized block design including total-tract digestibility measurements (dry matter (DM), starch (ST), crude protein (CP), Neutral-Detergent-Fibre (NDF)). The ration was supplemented with 15% of one of the treatments: ground corn, crushed wheat, sodagrain, ground barley, crushed barley, rolled barley. Chemical composition of faeces and feeds, individual feed intake, rumen pH (smaXtec®), and performance were measured. The APS was calculated from particle fractions which retained on eight sieves. Ruminal degradation rates (kd, %/h) for DM, ST and CP of grains were evaluated in sacco. Passage rate (kp %/h), based on APS, was used to calculate bypass fractions (%B[fraction]). Total digestible bypass fractions (%TB[fraction]) were calculated from intestinal digestibility of DM, ST and CP. Feed intake was lower in crushed wheat. Performance remained unaffected. Rumen pH parameters were not different between crushed and NaOH treated wheat. Apparent total-tract digestibility and kd of CP and ST were significantly lower for sodagrain when compared to crushed wheat resulting in an increased %BST of 19.2% and %BCP of 35.8% (crushed wheat 6.8% and 15.4%, resp.). Corn and sodagrain supplied the highest TBST and TBCP followed by barley and wheat. Due to the highest APS among treatments, sodagrain was characterized by the lowest kpST corresponding to an increased digestibility of NDF. NaOH treatment of wheat is recommended to increase bypass fractions. The use of crushed barley and corn is preferred over that of ground barley and wheat to prevent rumen acidosis. APS of grains can be used to calculate associated passage rates and nutritional values.

P4

Valorisation of microalgae sidestreams in poultry feed

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Microalgae are an emerging production system for high-quality endproducts such as mycosporine-like amino acids (MAAs). These MAAs have several applications due to their UV-absorbing and therapeutic properties. After deriving the MAAs, the biomass can be repurposed in poultry feed for a circular biosystem. Currently the main protein sources in chickenfeed include oil and legume seed. Microalgae biomass contains compounds (pigments, unsaturated fatty-acids) that might augment health-promoting effects on the chickens or their endproducts (meat and eggs). A problem that might occur are digestibility problems of these algae since they have a rigid cell wall. Therefore, several techniques were tested to perforate the cell wall and to investigate if nutrient digestibility was influenced by this intervention: freezing for 1 week at –20°C, as a powder and as a 10%-solution and Pulse Electric Field were tested. Both autotrophic and heterotrophic, processed and non-processed algae were stained with SytoxGreen after processing and disruption efficiency was determined with fluorescence microscopy. First results show a percentage (95%-CI between brackets) of broken cell walls in the purchased Chlorella biomass of 7.94% (5.91-9.97) and 11.58% (5.48-17.69) for autotrophic and heterotrophic Chlorella respectively. The PEF-processed algae show a disruption efficiency of 79.20% (73.60-84.80) (auto) and 83.90% (79.97-87.82) (hetero). An untreated living culture showed an efficiency of 1.55% (0.77-2.34). Frozen algae gave 7.33% (4.95-9.70) and 3.86% (2.75-4.98) (autotrophic and heterotrophic respectively). Since PEF is the most efficient technique, the PEF-processed algae are tested in digestibility trials. Therefore, different feed formulations with algae are compared for their digestibility by analysing feed and feces on protein, fat, crude cell and energy content.

P5

Optimisation of an *in vitro* batch fermentation model to compare the fermentation of dietary fibre by the porcine microbiota

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In vitro batch fermentation can serve as an important technique to measure the efficacy of dietary fibres such as prebiotics, or to compare the properties of different microbiotas. Yet, there are many possible variations, many of which rely on sophisticated pieces of equipment such as anaerobic chambers. We sought to compare techniques to optimise a simple method that would allow the comparison of different microbiotas and prebiotics. We hypothesised that the use of different buffers and pHs would affect the outcome of the *in vitro* fermentation. Throughout the optimisation process, we tested multiple buffers, different pHs and the use of resazurin for inocula from different gastrointestinal regions and from different herds of pigs and wild boar. These were tested alongside a range of prebiotics including inulin, chicory, commercially-available galactooligosaccharides and lactulose. For each fermentation, gas production was measured to indicate the rate of fermentation while final pH demonstrated buffering capacity. Short-chain fatty acids (SCFAs) were also measured by gas chromatography, and aliquots of fermentation cultures were grown on Petri dishes to compare colony-forming units of Lactobacillus and Bacillus. As a result, we established an effective protocol using gas-flushing to achieve anaerobic conditions, and found Menke's buffer to be an effective fermentation medium which buffered well to allow optimal microbial growth and thus fermentation and SCFA production. As such, we were able to compare fermentation profiles for different pigs and wild boar and show that there were differences in fermentation profiles according to the microbiota-type. This work provides scope for future investigations exploring the fermentation of dietary fibre by constituents of the porcine microbiota.

P6

Moisture content of wheat straw affects *C. subvermispora* and *L. edodes* growth and delignification

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Ruminants can utilize lignocellulosic biomass as an energy source since they have a unique digestive system where rumen microbes can degrade structural carbohydrates, such as (hemi)cellulose. However, lignin in the cell walls limits rumen microbes to more completely degrade structural carbohydrates, resulting in a lower feeding value. White-rot fungi can selectively degrade lignin and improve the degradability and nutritional value of highly lignified biomass. This study aimed to determine the growth speed of *Ceriporiopsis subvermispora*, and *Lentinula edodes* on wheat straw at different moisture contents and examine the changes in nutritional quality. (I) growth speed of *C. subvermispora* and *L. edodes* on wheat straw at different moisture contents (38, 49, 59 and 69%) was determined in race tubes over 2 weeks. *C. subvermispora* could colonize wheat straw with moisture contents higher than 49%, with the growth rate at a moisture content of 69% being faster ($P<0.05$) than that of 38 and 49%. *L. edodes* could only colonize wheat straw with a moisture content at 69%, but was slower ($P<0.05$) than *C. subvermispora* at the same moisture content. (II) wheat straw with various moisture contents (41, 51, 62, 70 and 77%) were incubated with *C. subvermispora* and *L. edodes* for 8 weeks. *In vitro* gas production (IVGP) and chemical composition were determined after 0, 4, 6, 7 and 8 weeks of incubation. Both fungi increased ($P<0.05$) IVGP and cellulose content as well as decreased ($P<0.05$) lignin and hemicellulose content when moisture content was 77%. *C. subvermispora* and *L. edodes* could colonize when moisture content was higher than 50 and 70% respectively, but delignify only when moisture content was higher than 70%. The nutritional value of wheat straw for ruminants was only improved by fungi at the highest moisture content (~77%).

P7

Wound-healing assay for nutritional compounds: not a “one-size-fits-all” method

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The wound-healing assay is a simple, easy and inexpensive method to monitor cell migration *in vitro*. A scratch is made in a monolayer of cells and the wound closure rate is determined by comparing different images over time. Since this study aims to determine the effect of several short-chain fatty acids (acetate, butyrate and propionate) in the context of pig feed, a porcine intestinal cell line, IPEC-J2, was chosen. These cells display microvilli on their apical side and tight junctions sealing neighbouring cells together. However, usually these cells are being cultured in foetal bovine serum (FBS, 5%) which make them more robust, while supplementation with porcine serum (PS, 5%) results in characteristics that mimic the in vivo situation much better. Additionally, when studying the effect of nutrients, it is advisable that the negative control is not too good since this might mask beneficial effects of your studied compound. Therefore, an additional “starvation step”, where the cells are being exposed to a less rich medium (1% PS) can increase the gap between the studied compounds and the negative control. Linked to this, a positive control is equally important. The epidermal growth factor (EGF) was suggested by literature, however this compound promotes proliferation, while propionate for example, is more associated with cell migration, hence a compound that promotes cell migration rather than proliferation should be preferred. Spermidine was proven to be effective as a positive control in our protocol ($p = 0.033$), while EGF (5 ng/ml or 50 ng/ml) failed ($p = 0.517$ and $p = 0.939$, respectively). Propionate at a 2 mM concentration showed to improve wound healing significantly ($p = 0.049$). A mixed model was fitted including plate as a random effect. The assays for acetate and butyrate are still ongoing.

P8

Using a dynamic and a static *in vitro* simulation model of the avian foregut to evaluate pre-cecal digestion of sustainable protein-rich feed ingredients

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Inclusion of alternative protein sources (APS) as SBM substitute in broiler diets is expected to have a crucial impact in securing a more sustainable meat production in the near future. Environmental impact can be reduced by implementation of protein-rich feed ingredients, which are either locally produced, fit in circular agricultural systems or are of innovative nature and can be produced highly efficient, but require further innovation for a more intensive usage. A selection of eight 'highly potential' APS is made in an earlier research phase, by combining results from extensive literature study and stakeholder conversations with nutritionists from animal sciences and feed industry. This selection includes: lupins, field beans, rapeseed meal, potato protein, blood meal (porcine), black soldier fly larvae meal, micro algae (Chlorella sorokiniana) and single cell protein. Nutritional and environmental properties of the selected APS are studied in the different phases of this PhD track. In all phases Brazilian GMO SBM is included as a reference. In this *in vitro* study a dynamic *in vitro* model - 'Chicken Alimentary Tract *In vitro* Model' (CALIMERO), adaptation to TNO Intestinal Model (TIM) – as well as a static *in vitro* model - adaptation to the model described by Moquet et al. (2018) – are used to simulate pre-cecal protein digestion of the selected APS in the foregut of broiler chicks. A high protein digestibility secures good technical results and an optimal gut health status. After digestion samples of the digesta are collected from both models and laboratory methods are applied to detect nitrogen fractions. Analytic results are used to calculate protein digestion coefficients. During the ANR 2023 used methodology and preliminary results of the *in vitro* analyses in both models will be presented.

P9

Classifying lipogenic and glucogenic diets in dairy cows based on metabolomics profiles

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The objective of this study was to differentiate and explore the effect of diets on early lactation dairy cows based on plasma metabolome data. Holstein-Friesian cows were randomly assigned to a glucogenic (n=15) or lipogenic (n=15) diet in early lactation. Plasma was collected in week -2, 2, and 4 relative to calving. Plasma metabolomics profiles were detected using LC-MS. A total of 37 metabolites were identified. For each cow per timepoint two dataset were available. 1. Extinction values for metabolites (extinction dataset); and 2. Ratios between extinction values for metabolites (ratio dataset). Classification of cows to the two diets was done using the XGboost algorithm. The ratio dataset resulted in better classification performance compared with the extinction dataset for cows fed a lipogenic and a glucogenic diet. Model performance as measured by the area under the ROC curve (AUC) of lipogenic and glucogenic treatment improved from 0.606 to 0.753 and from 0.696 to 0.842 in week 2 and 4, respectively. The top features to classify the lipogenic and glucogenic treatment were the ratio of arginine to tyrosine and the ratio of aspartic acid to valine in week 2 and 4, respectively. For cows fed the lipogenic diet, choline and the ratio of creatinine to tryptophan were top features to classify cows in week 2 and 4. For cows fed the glucogenic diet, methionine and the ratio of 4-hydroxyproline to choline were top features to classify cows in week 2 and 4. Carnitine and the ratio of asparagine to carnitine were top features to classify week -2 and 4 both for cows fed a lipogenic or a glucogenic diet. This study shows that the ratios of metabolites better classify diets in dairy cows in early lactation compared with absolute extinction values.

P10

Reference diets containing crystalline amino acids at 120% of requirements for adult dogs is not appropriate for estimating protein quality of ingredients used in extruded diets

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The indicator amino acid oxidation (IAAO) method is used to determine metabolic availability (MA) of AA in food. Ingredients are compared to a reference diet of AA assumed to have 100% MA. Pulses are suggested to have poor methionine (Met) MA, but no data exists. Therefore, we sought to quantify the MA of Met in peas and chicken meal (CM) compared to a reference diet. A partially replicated 7x7 Latin square design using 9 adult dogs (26.7kg+/-2.7kg BW) received: BAS: reference providing Met at 19 (BAS0.10), 40 (BAS0.21), and 63% (BAS0.33) of requirement, CHK: CM-based and PEA: green pea-based diets, both providing Met at 63% of requirement. Treatments were created by blending BAS0.10 with PEA (BAP) or CHK (BAC) generating diets with Met at 40% of requirement. This created 3 graded levels of Met for CM, peas, and BAS reference diet permitting a slope ratio approach. After 2 (D2) and 6 (D6) days diet adaptation per period, IAAO was performed. Fasting volume of expired CO2 (VCO2) measurements were taken, then dogs were fed 13 small meals. Meal 6 contained a priming dose (9.4mg/kg BW) of L-[1-13C]-Phenylalanine (Phe) followed by a constant dose in meals 6-13. Breath samples were collected and enrichment of 13CO2 was measured using LC-MS/MS to calculate oxidation (F13CO2/kg BW/h). Data was analyzed using proc GLIMMIX with dog and period as random effects, and diet, %Met, IAAO day, and their interactions as fixed effects. There was no effect of day on MA. CM had the best response and pea was 66% and 51% MA of CM on D2 and D6. BAS had no response suggesting another limiting AA in the BAS diet and peas have lower MA than CM.

P11

Fermentation of endogenous protein in the colon of pigs determined by a gas production technique

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Protein fermentation in the hindgut of pigs is often regarded as harmful to health. One of the strategies to reduce the negative impact is to limit protein intake. However, not only dietary protein but also endogenous protein (EP) may contribute to fermentation as it enters the hindgut. Therefore, the fermentability of porcine EP was investigated. Ileal digesta samples from pigs fed nitrogen-free diets in 5 different studies, colonic mucus and whey protein isolate (WPI) were tested in an *in vitro* gas production system with porcine faecal inoculum. Nitrogen limited microbial activity through the use of a nitrogen-free buffer and an excess of fermentable carbohydrates with gas production (GP) recorded for 48 h. Compared to most of the digesta samples, mucus had a greater maximum gas production rate (Rmax, 25.0±1.96 vs 18.5±4.01) and shorter time to reach its maximum rate (Tmax, 5.0±0.61 vs 7.2±2.77), while WPI as intact protein showed the lowest Rmax (12.2±2.30) and highest Tmax (12.9±4.99). Differences in Rmax and Tmax were also found between different digesta samples ($p<0.05$). Subsequently, we tested the hypothesis that smaller molecular proteinaceous structures are easier to ferment. Indeed, WPI mixtures with a higher degree of hydrolysis showed a higher Rmax. Also, size-exclusion chromatography showed in the endogenous samples from pigs, Rmax = 0.3 × [Quantity of molecule 0-1 kDa] + 0.6 × [Quantity of molecule >50 kDa] ($p <0.05$). Furthermore, we tested the fermentation potential of ileal digesta from pigs fed different protein ingredients, which also showed a similar relationship. Altogether this research provides a first understanding of the fermentation potential of protein in pigs.

P12

Defining the ideal growth tract of the modern Holstein Friesian heifer in Flanders

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Aiming for an earlier age at first calving (AFC) supports a more economical and ecological farming. To accomplish that, optimal rearing management is crucial to maintain optimal growth per age. However, in the last 30 years, Holstein Friesian heifers have evolved to become more productive, so available growth curves and weight-based nutrient requirements are not up to date anymore. Therefore, body weight (BW), heart girth (HG) and hip height (HT) of Holstein Friesian heifers were monitored quarterly from birth until 1 month before calving on 14 selected Flemish farms to evaluate the ideal growth tract. The farm selection was based on: 1) yearly milk production > 10 000 kg per cow; 2) AFC < 24 m and 3) calving interval < 400 d. In total, 1 921 heifers were monitored and led to 10 116 BW, 8 792 HG and 9 864 HT measurements. These growth performances resulted in an average growth tract that followed the growth curve of AFC of 22 m . Indeed, heifers achieved the ideal weight for artificial insemination of 400 kg at 13 m (HG= 175 cm; HT= 137 cm). At this age, based on NRC 2021 and daily feed intake (DFI) data collected from ILVO, heifers should have a DFI between 8.6 and 9 kg DM with energy and protein requirements of 6 890 VEM and 580 DVE, respectively. At calving, the weight of the heifer (incl. calf) should be 630 kg (HG= 206 cm; HT= 152 cm). In conclusion, to achieve an AFC <24 m in practice, management and more specifically nutrition should be adapted to the growth curve for AFC 22m.

P13

Inter-animal variability in systemic inflammation status in early lactation and its relation to the cows' metabolic status and reproductive performance

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Around parturition, inflammation occurs in almost all cows. Generally, this situation resumes within the first week after calving, while a prolonged inflammation characterized by elevated/decreased levels of positive/negative acute-phase proteins (APP) may be detrimental. Unfortunately, variations in basal blood APP concentrations among herds hamper the identification of critical blood APP thresholds to distinguish dairy cows encountering prolonged inflammation. Hence, multivariate clustering could be a way to identify animals differing in inflammatory status. In this experiment, 99 individual transition dairy cows and 109 observations were involved, following the same transition management. Blood was taken at -7, 3, 6, 9 and 21 days in milk (DIM) and glucose, BHBA, NEFA, insulin, IGF-1 and fructosamine were analyzed. Additionally, APP including haptoglobin (Hp), serum amyloid A (SAA) and albumin-to-globulin ratio (A:G) were determined in the blood of 21 DIM. Diseased cases (n=20) were removed from the dataset and treated as a separate group. The three APP parameters were used in a k-medoids clustering and cows were grouped into two categories, i.e. acute phase response (APR, n=39) vs non-APR (n=50). In the non-APR group, the highest A:G and lowest concentrations of SAA were observed compared with the other groups ($P<0.05$). In addition, the non-APR group showed a more balanced metabolic status, characterized by the highest IGF-1 level among the three groups and higher fructosamine than the APR group ($P<0.05$). The median of days open did not differ between groups. A higher culling rate was noticed in the APR than non-APR (51% vs. 28%, $P<0.05$). These findings suggest that cows vary in the resolution of inflammation at 21 DIM and the ones which have an enhanced inflammation show more severe metabolic stress.

P15

Supplementation of dried fermentation solubles may result in an increased post-weaning performance in piglets

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Supporting gut health of weaned piglets with nutrition may prevent a post-weaning dip. Such a nutritional intervention may be the addition of dried fermentation solubles (DFS) (Bacillus licheniformis) to a weaner diet. It was hypothesized that these DFS may act on the process of protein digestion, resulting in an improved performance after weaning. In this trial, 376 weaned piglets (TN70 x Tempo) at the Denkavit Innovation Centre received either a low or high crude protein weaner diet with or without addition of 130 ppm DFS on-top the first two weeks after weaning. Thereafter all groups received the same rearing diet from day 15 until day 36. Piglets were weighed individually at weaning and weighed per pen at day 8, 15 and 36. Feed intake was registered per pen at day 8, 15 and 36. Faecal colour and consistency were noted at day 5, 8, 12, 15, 19, 22, 29 and 36. Faecal calprotectin levels were measured on day 12 for 16 randomly selected piglets per treatment. Data was analysed with an ANCOVA model with weaning weight as covariate. Weight at day 15 (11.5 vs. 12.1 kg/piglet; $P<0.01$), weight gain from day 1-15 (2.8 vs. 3.4 kg/piglet; $P<0.001$) and feed efficiency from day 1-15 (0.72 vs. 0.80; $P=0.001$) were significantly higher for piglets fed a high protein diet with DFS compared piglets fed a high protein diet without DFS. No significant effect on performance was found between groups for the addition of DFS to a low protein diet. Both groups with the addition of DFS showed less loose and grey faeces. Calprotectin levels did not significantly differ between treatment groups. The effect of DFS on performance after weaning was thus dependent on protein level in the diet, whereby the addition of DFS to a high protein level resulted in an increased performance post-weaning.

P16

Non-invasive measurements: Saliva sampling around parturition in sows

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Insights in mode of action during animal studies often require invasive measurements. Saliva sampling however is non-invasive. In saliva, haptoglobin can be measured as an indicator for inflammation and acute stress. It is hypothesised that sows experience increased acute stress and inflammation post-parturition, changes in haptoglobin levels were measured to evaluate the levels of acute stress and inflammation around parturition in sows. Salivary haptoglobin levels were determined of 134 sows farrowing without any form of birth assistance. Per sow, one to two saliva samples were collected 24-48h pre-parturition and one 24-96h post-parturition by sows voluntarily chewing on a presented cottonwool. Parturition was recorded using camera's, so exact onset of parturition in relation to time of sampling could be calculated. Differences in haptoglobin levels pre- and post-parturition among sows were evaluated using PROC MIXED in SAS (version 9.4). Treatment, time of sampling and difference between the two sampling moments were added as random effects, while parity class (1=parity1+2; 2=parity3+4; 3=parity>4) was added as a fixed effect. Results showed, salivary haptoglobin levels were higher post- compared to pre-parturition. No difference in pre-parturition haptoglobin levels were observed between parity classes. Parity class 1 sows showed a significant larger increase in haptoglobin level between pre- and post-parturition (P=0.04) compared to parity class 2 and 3. Indicating that younger sows experience more stress during- and post-parturition. Haptoglobin measured in saliva around parturition is a promising non-invasive measurement for stress since it shows clear differences in haptoglobin levels among sows of various parities. However, a high inter-individual variability was observed in basal salivary haptoglobin levels, which likely makes it more difficult to detect smaller differences. Saliva samples were easy to collect, causing no stress for the animal making it an convenient option for evaluating stress physiology in pigs for animal nutrition research.

P17

Productive responses of broilers fed diets with different feed compositions during heat stress

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Heat stress can adversely affect broiler performance and meat quality. Dietary composition could potentially mitigate those negative effects. Diet-induced thermogenesis is mainly determined by the protein fraction and increasing dietary fat can stimulate nutrient absorption and provide extra metabolizable energy (ME). Therefore, we studied the effects of reduced protein and/or increased dietary fat on performance and meat quality during heat stress. In total, 1920 male Ross 308 broilers were divided in 8 treatments (8 replicates/treatment, 30 birds/replicate) according to a 2 x 4 factorial design, i.e., birds were kept in thermoneutral conditions or subjected to heat stress (32°C ± 2°C and RH 55–65% for 6h daily, d29-43) and 4 finisher diets (d26-39) were formulated. The diets were: control, standard broiler diet (ME 12.2 MJ/kg, 18.2% crude protein (CP) and 8.0% crude fat (CF)), low CP diet (LCP) (CP 17.3%), low CP and high CF diet (LCPHCF) (ME 12.6 MJ/kg; CP 17.3%; CF 9.7 %), and high CF diet (HCF) (ME 12.5 MJ/kg; CP 18.2%; CF 9.6%). Statistical analysis was done with RStudio using a generalized linear mixed model. Heat stress had negative effects on performance: growth ($p<0.01$), feed intake ($p<0.01$), final weight ($p<0.01$), feed conversion ($p<0.01$), and mortality ($p<0.01$). Interestingly, broilers in heat stress had significantly higher slaughter yield ($p<0.01$), upper thigh% ($p<0.01$), drumstick% ($p<0.01$), and breast muscle% ($p<0.01$). The different feed compositions did not significantly improve broilers' performance during heat stress in this experimental design. However, the amount of protein reduction and lower feed intake during heat stress are not to be excluded as reason for this lack of effect.

P18

Bac*in*2DLiver

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Equine metabolic syndrome (EMS) is not a disease but a series of risk factors that can result in the painful clinical outcome of laminitis. Excessive grass consumption on high-fructan pastures can exacerbate EMS, prompting pasture-associated laminitis. Fructan fermentation is predominantly associated with dysbacteriosis in the equine hindgut and the alteration of microbial produced fermentation end-products and metabolites that are later metabolised by the liver. However, little is known about how these alterations following fructan overload impact the metabolic and immunological status of the liver. The use of animals in experimentation is under scrutiny. Organoids are 3D mini-organs that can preserve genetic and epigenetic information of the native tissue, making them an ideal tool for studying disease. Therefore, new equine *in vitro* models are essential for future investigations. Bac*in*2DLiver is a study that aims to combine *in vitro* techniques to develop a tool that models the gut-liver axis to study the impact of bacterial fermentation on cellular and metabolic responses *in vitro* using 2D liver organoids. By utilising the *in vitro* gas production technique, grass fructans, as well as other carbohydrate sources or compounds, can be fermented in equine digesta and sampled at various time points to assess the types and ranges of fermentation end-products and metabolites present. The effect of end-products and metabolites on (liver) metabolism can be studied by applying the collected fermentation fluids to equine-based organoids and analysing the changes in gene expression. Furthermore, by co-culturing liver organoids with liver resident macrophages (Kupffer cells), the model will provide new insights into the immunological response of the

liver following exposure to various metabolites. This model that combines these two *in vitro* techniques would be a valuable and innovative asset for studying EMS in the future.

P19

Assessment of the bioavailability of collagen hydrolysates after oral intake in dogs

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Introduction- Osteoarthritis (OA) affects 3%-7% of dogs worldwide(1). Collagen hydrolysates(CH) were shown to reduce joint pain and improve patient mobility in human OA(2), but its effects vary largely between studies in dogs(3,4). The bioavailability of CH in dogs is unknown and most used CH doses were extrapolated from studies in people. Based on an extrapolated dose from people, at least 250 mg/kg CH is expected to significantly increase plasma concentrations of hydroxyproline (Hyp), a representative marker, with peak concentrations occurring after 60 minutes.

Methods- To determine the effects of single CH-intake on plasma Hyp concentrations, three dogs were supplemented CH (Hydro-P Premium, Darling Ingredients) in incremental dosages (0, 250, 500, 750 mg/kg) on top of normal food intake. Three day-washout was used between dosages. Plasma Hyp levels were measured at 0, 30, 60 and 90 minutes after CH intake with liquid chromatography-mass spectrometry, and analyzed using Friedman tests.

The effects of repetitive CH intake on Hyp baseline and peak concentrations were determined by administering CH for 7 days to eight healthy beagle dogs in incremental dosages (0, 250, 500, 750 mg/kg) in a randomized order with 7 day-washout between doses. Plasma Hyp was measured at 0 and 60 minutes and analyzed using mixed models.

Results and discussion- Peak concentrations of Hyp occurred 60 minutes after intake ($P<0.001$). Repetitive intake of 250, 500 and 750 mg/kg CH significantly increased Hyp concentrations over controls ($P<0.001$ for each dosage). No additive effect of 750 compared to 500 mg/kg was found ($P=0.2496$). Baseline Hyp concentrations were not affected by dosage. Using this data, the clinical efficacy of CH on dog OA patients will be investigated in a future study using a dose of 500 mg/kg CH.

[1] Anderson et al., 2018, Sci Rep, 8 [2] Kumar et al., 2015, J Sci Food Agric, 95 [3] Beynen et al., 2010 Am J Vet Sci, 5 [4] Böswald et al., 2022, ESVCN

P20

Effect of lysine level in finisher diets on performance in crossbreds from two terminal sire lines

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Lysine requirements in low-protein diets, impacting feed costs and nitrogen excretion, depend on sex and genotype due to differences in lean growth and feed intake capacity. The current study evaluated the effect of lysine level in the finisher phase (80-115kg) on growth performance in crossbred pigs from two terminal sire lines. Gilts and barrows (n=360) with a different paternal genetic background (stress negative sire line selected for growth rate versus stress positive sire line selected for carcass quality) were allocated to a isocaloric low or high lysine diet (6.3g versus 7.8g SID lysine/kg diet, with 12.6% and 14% crude protein), according to a 2×2×2 factorial design. The feed conversion ratio (FCR) was lower for gilts compared to barrows (2.85 versus 2.93, $p=0.049$). Within sexes, there was only a significant difference in FCR between the low and high lysine diet of the crossbred selected for carcass quality (2.95 versus 2.76, $p=0.023$). Higher serum urea levels in barrows demonstrated the lower amino acid requirements compared to gilts ($p<0.001$). In barrows, the high lysine diet resulted in 31% higher serum urea compared to the low lysine diet ($p<0.001$), independent of sire line, suggesting an excess of protein in the high lysine diet for all barrows. In gilts, a trend for higher serum urea (9% increase, $p=0.06$) was observed for the high compared to the low lysine diet, also independent of sire line. In general, both the low lysine diet and the genetic background selected for carcass quality positively affected nitrogen efficiency in the finisher phase. Only in gilts selected for carcass quality, the low lysine diet did not result in a better nitrogen efficiency compared to the high lysine diet.

P21

In vitro evaluation of the methane reducing potential of extruded linseed in relation to roughage composition of dairy cattle

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Mitigation measures are required to reduce the impact of ruminants on the climate, with feeding strategies being the most promising on the short term. Results of previous in vivo experiments suggest a reduced effectiveness of extruded linseed (EL) in diets with higher proportions of a more fibrous roughage source, such as grass silage (GS). The main objective of this *in vitro* experiment was to evaluate the effectiveness of EL to reduce enteric CH4 emissions in diets with increasing proportions of GS. Five GS-MS-mixtures with varying ratios of GS to MS (0:100, 25:75, 50:50, 75:25 and 100:0) were formulated. To these mixtures pressed beet pulp (10% of total DM) and concentrates (35% of total DM) were added. The concentrate either (TRT) or not (CTRL) included an EL product, which proportionally replaced 16.8% of the other concentrates. Five CTRL and TRT concentrates were composed to formulate total diets with similar net energy, protein digestible in the small intestine, starch, sugar, crude protein and NDF. Secondly, the CTRL and TRT concentrates of the 50:50-GS/MS-diet were used in combination with all GS/MS-ratios. All substrates (n=20) were incubated in triplicate for 24h in an *in vitro* batch system and each run was repeated 3 times. After incubation, pH, gas pressure (kPa), CH4 production (µmol/flask), volatile fatty acids (VFA, µmol/flask), and organic matter degradability were measured. None of these parameters were affected by EL. Additionally, neither variation in GS proportions nor in chemical composition of the concentrate affected any of the parameters. Further experiments need to be performed to gain more insight in this mitigation strategy and the suitability of a single-end-point batch *in vitro* approach to evaluate this strategy in relation to the dietary roughage composition.

P22

Effects of extending lactation for dairy cows on health, development and production of their calves

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Extending the voluntary waiting period for insemination (VWP) in dairy cows is of interest to reduce the frequency of calving events and inseminate at a moment with less fertility problems. Little is known about the impact of extension of the dams' VWP on the calf. The aim of this study was to evaluate the effect of extending VWP of dams on growth, metabolites, and production performance of their offspring. Holstein Friesian dairy cows (n=154) were blocked according to parity, milk yield, somatic cell count and randomly assigned to a VWP of 50, 125, or 200 days. For the current study, Holstein-Friesian heifer calves (n=61) from cows with different VWP were monitored from birth until 100 days in milk after their first calving. Birth weight did not differ among heifer calves of the 3 VWP groups. During the rearing phase, body weight (BW) of heifers was not different among VWP groups. During the first 100 DIM, heifers in VWP50 had a greater BW (557 kg, P<0.01) and a greater fat and protein corrected milk yield (FPCM, 29.01 kg/d, P<0.01), compared with heifers in VWP125 (BW: 533 kg; FPCM: 27.04 kg/d). When heifers were regrouped according to their mothers' real calving interval (CInt; CInt_1: < 409 days; CInt_2: 409-468 days; CInt_3: >468 days), heifers born to mothers with CInt_3 had a greater BW after calving compared with heifers in CInt_2 (559kg vs. 537kg, P=0.01). Heifers in CInt_1 had higher FPCM than heifers in CInt_2 (29.13 kg/d vs. 27.23 kg/d, P<0.01). In conclusion, extending VWP of dams did not affect growth of heifer calves during the rearing phase, but affected body condition and milk performance of heifers during the start of their first lactation.

P23

Variations in bulk milk urea content on dairy farms in Flanders, Belgium in 2019-2021

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Urged by environmental concerns and legislation, Flemish dairy farmers are in desperate need of methods to reduce their ammonia emissions. One way of reducing ammonia emissions is by reducing the amount of crude protein in the ration of dairy cattle, leading to a lower urinal urea excretion. To prove as a farmer that you are feeding low-protein diets, diet composition should be regularly monitored and controlled. However, today, that is not the case. Alternative ways of evaluating the protein content of the ration are therefore needed. Milk urea could be used to this end, since milk urea content is known to be largely driven by the protein content of the ration. In this research, we determined the inter- and intrafarm variability in bulk milk urea content on Flemish dairy farms and evaluated its potential as a predictive tool for the protein content of dairy rations. We analysed data from Milk Control Centre Flanders (MCC) from 1,652,470 milk collections from 3,881 Flemish dairy farms throughout 2019, 2020 and 2021. Mean bulk milk urea content over these three years was 229.6 ± 51.8 mg/L, with higher average values in summer (244.6 ± 53.1 mg/L in August) compared to winter (208.0 ± 51.9 mg/L in December), and brief peaks in milk urea content as a response to heatwaves. However, most of the variation of the milk urea content on individual farm level could not be explained by known variables such as date or temperature. We conclude that milk urea content cannot be used as a standalone predictive tool for the protein content of dairy rations. Further research will determine which additional parameters or analyses are needed to easily monitor the protein content of the ration in a cost-effective, practical and reliable manner.

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Effect of particle size and dry matter content of a total mixed ration (TMR) on rumen content mass and compostion, fluid dynamics and volatile fatty acid production of lactating dairy cows

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To study the trade-off between increased dry matter (DM) intake and decreased rumen volatile fatty acid (VFA) production upon a reduction of ration particle size, eight rumen cannulated lactating dairy cows were used in a cross-over study where either a coarse or fine, but nutritionally identical, TMR was fed. A fine TMR (34% DM and 15.8% of DM ≥ 8 mm screen) was hypothesized to increase DM intake but decrease VFA yield per kg DM compared with a coarse TMR (48% DM and 30.1% of DM ≥ 8 mm screen). Using the valerate-cobalt technique, rumen volume, fluid passage rate and VFA clearance rate were measured. Subsequently, VFA production was calculated. Finer TMR particle size increased DM intake (27.5 vs 25.9 kg/d, $P < 0.01$), but did not affect rumen content mass (118 kg, $P = 0.45$) and rumen fluid mass (100 kg, $P = 0.45$) indicating increased feed passage rate. Rumen VFA concentration (108 mM; $P = 0.15$) and fractions of acetic (63%), propionic (21%), and butyric acid (16%) were not affected ($P \geq 0.71$) by TMR particle size, indicating ration composition is the main driver for these variables. Likewise, fractional VFA clearance (0.57 /h), fluid passage (0.24 /h) and VFA absorption (0.33 /h) rates were not affected ($P \geq 0.22$) by TMR particle size, indicating that feed particle size did not affect rumen fluid dynamics. Contrary to expectations, yield of VFA (5.0 mol/kg DM, $P = 0.45$) was not affected by TMR particle size, suggesting the increase in feed passage rate was insufficient to affect fermentation efficiency. This was, however, not expressed in a greater VFA production (135 mol/d, $P = 0.28$) despite the greater feed intake with a fine TMR. In conclusion, the coarse or fine TMRs did not affect rumen fermentation performance in the conditions under study.

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