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- *Review Lecture (Übersichtsreferat)*
- *Abstracts (Kurzfassungen der Originalmitteilungen)*

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Contributions made by the members of the reviewer panel are gratefully acknowledged.

J.R. Aschenbach
Chairman

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15:00		Henneberg/Lehmann Foundation Awards Ceremony (separate invitation)	
17:00		End of conference	

		Undesirable substances and antinutrients	page
9:00		88. Investigation of the rumen digesta washing mechanism in live cattle **Valerio S., Hummel J., Codron D., Hatt J.-M., Clauss M. – Zurich / Goettingen / Bloemfontein*	112
9:15		89. Transfer of pyrrolizidine alkaloids (PAs) into milk in a chronic PA exposure scenario of dairy cows **Knoop K., Knappstein K., Gabler A., Klein L., Gottschalk C., Tänzer J., These A., Kaltner F., Meyer U., Saltzmann J., Dänicke S. – Braunschweig / Kiel / Munich / Berlin / Giessen*	113
9:30		90. The effects of microplastic on ruminal dry matter degradation *ex vivo* **Eichinger J., Kuenz S., Eckel F., Zollfrank C., Windisch W., Brugger D.– Freising / Straubing / Zurich / Posieux*	114
9:45		91. Comparative ruminal metabolism of pyrrolizidine alkaloids in sheep and cattle **Tänzer J., Knappstein K., Lamp J., Dänicke S., Saltzmann J., Knoop K., Gottschalk C., These A. – Berlin / Kiel / Braunschweig*	115
9:48		92. Oral intake of sycamore seedlings by grazing dairy cows on pasture and detection of Hypoglycin A metabolites in milk **Engel A. M., El-Khatib A., Klevenhusen F., Aboling S., Weigel S., Pieper R., Fischer-Tenhagen C. – Berlin / Witzenhausen / Hanover*	116
9:51		93. Determination of Mineral Oil Hydrocarbons in calves and piglets milk replacer by LC-GC-FID and GC×GC-ToF-MS **Yang C., Kruschinski S., Teltewskoi M., Luch A., Pieper R., Steinhoff-Wagner J., Roloff A. – Berlin / Freising*	117

09:54	Poster discussion – Undesirable substances and antinutrients
10:15	Coffee and Conversation break
10:30	*See Lecture hall 008*

Review Lecture

"Novel Feed and Novel Food - Hype or Real Future?"

"Novel Feed und Novel Food – Hype oder reale Zukunft?"

Visscher C., Wilke,V. – Hanover

Food production has to be optimized in terms of environmental impacts and food security for the world's growing population. Thereby, changes in food production inevitably affect the feed production chain. The ecological footprint of food differs depending on the type, process, and geographic region of the production (Van Raamsdonk et al. 2023). In this context, livestock production is one of the most controversial human food production chains due to its large environmental impact. Therefore, in addition to the potential of Novel Foods to improve health, a particular focus will be on evaluating the potential of Novel Foods and alternative feeds to close the protein gap while meeting sustainability goals.

Terms and Definitions

On the websites of the European Union (EU), Novel Foods are defined as foods that have not been consumed by humans in significant quantities in the EU before May 15, 1997, when the first Novel Food Regulation came into force. In this context, Novel Foods can have various origins. They can be newly developed, innovative foods, foods produced using new technologies and production processes as well as foods that are or have traditionally been consumed outside of the EU. Examples are extracts of existing foods (Antarctic Krill oil rich in phospholipids from Euphausia superba), agricultural products from third countries (chia seeds, etc.) or foods from new production processes, e.g., UV-treated foods (European Commission 2023a). These Novel Foods must be safe for consumers, properly labeled and – if the Novel Food is intended to replace another food, it must not differ in such a way that its consumption would be nutritionally disadvantageous for the consumer (European Commission 2023a). In addition, pre-market approval of Novel Foods is required on the basis of an evaluation in accordance with the principles just mentioned (European Commission 2023a). The relevant legal basis is Regulation (EU) 2015/2283; the European Food Safety Authority (EFSA) has updated and developed a scientific and technical guidance for the preparation and presentation of applications for authorization of Novel Foods (EFSA 2016). Article 3 of Regulation (EU) 2015/2283 lists the relevant categories under which Novel Foods must be able to be classified. The notifications and the applications can be found on the corresponding pages of the European Commission (European Commission 2023b). For example, only one application and two notifications are listed for 2022, but 29 applications and six notifications are listed for 2021.

When reviewing the Novel Food Catalog, it becomes clear that Novel Foods are more likely to represent specialties. Commonly alternative proteins and their sources including both animal-derived alternative proteins such as insects and cultured meat, and non-animal alternatives such as plants and algae. Basically, the orientation indicates that there is a political motivation to establish a market for Novel Foods that can be consumed directly by humans. Insects, which generally play an important role in Novel Foods, are also explicitly considered here. According to the Food and Agriculture Organization of the United Nations (FAO), insects as food are becoming a particularly important issue in the 21st century due to the rising cost of animal protein, food insecurity, environmental pressures, population growth and the increasing demand for protein among the middle classes (European Commission 2023b). In Horizon Europe, which is a funding program for research and innovation, insect-based proteins are considered as one of the key research areas (European Commission 2023b).

While the term “Novel Food” is clearly defined in the food sector, there is no comparable definition for “Novel Feed” in the European legal context. In the feed sector, there is currently neither a legal definition of the term “Novel Feed” nor a separate regulation is currently in force in the EU (OECD 2022). The situation is different in other parts of the world. For example, the Canadian Food Inspection Agency (CFIA) and Health Canada have published a joint webpage describing Canada’s regulatory framework for the environmental release of Plants with Novel Traits (PNTs), Novel Feeds, Novel Foods, and how products derived from gene editing techniques may or may not be considered novel. A complete list of approved Novel Feeds from plant sources is available online. The CFIA is responsible for the pre-market assessment of Novel Feeds under the Feeds Act and Regulations. To date, for example, the CFIA has approved over 140 Novel Feeds derived from plant sources and over 40 Novel Feeds from microbial sources (OECD 2022). This includes Novel Feeds such as herbicide-tolerant sorghum (S&W seeds) and dicamba tolerant maize (MON 87429) etc. (OECD 2022). This means that in particular genetically modified feedstuffs are also listed here. Assessments for some of these products are also carried out in the EU and can be found in the EFSA Journal (EFSA 2022).

Under Novel Feeds, the scientific community is certainly thinking more often about feedstuffs that have not existed on a large scale before, such as insects or algae. Nonetheless, the main question should always be, whether it is feasible that these sources are scalable, both economically and sustainably.

Future Protein demand

From a global perspective, the growing world population seems to require a further increase in the production of food of animal origin (van Zanten et al. 2016, Rauw 2022). However, livestock husbandry is one of the most disputed production chains of human food due to the large ecological footprint (Van Raamsdonk et al. 2023). In the context of a continually growing world population, agriculture is worldwide challenged by two major incentives: minimizing negative environmental impacts and maximizing food security (Van Raamsdonk et al. 2023). This development also increases the need to align existing processes and growth with efficiency standards. In the European context, it is the desire for greater sustainability that is driving this further development.

In the context of Novel Feeds, it is particularly interesting to look at protein supply in aquaculture because it has the widest range of ideas for new and alternative feeds. These include low-trophic level (LTL) species (mesopelagic fish, zooplankton, polychaetes, macroalgae, and crustaceans), novel microbial ingredients (bacteria, yeast, and microalgae), insects (black soldier fly, yellow mealworm, and crickets), animal by-products (poultry meal, meat and bone meal, blood meal, and hydrolyzed feather meal), and by-products from other commercial production (Albrektsen et al. 2022).

Current unexploited marine feed resources of significant biomass are found at lower-trophic levels, consisting mainly of populations of animal plankton, mesopelagic fish, and algae. However, the intensification of harvesting and cultivation of marine species, alone or co-cultivated with other marine species in integrated multitrophic aquaculture (IMTA), will require the use of large areas of sea and land, both of which need to be critically evaluated through appropriate impact studies (Albrektsen et al. 2022). Despite the underlying potential of functional food/feed formulation, extensive research, development efforts and impact assessments are still required before, for example, microalgae become a commercial reality in food and feed formulation as an alternative protein source (Lafarga and Acién 2022).

Microbial ingredients such as bacteria and yeasts, as well as insects, are increasingly being considered as promising alternatives due to their ability to convert organic non-food waste streams from forestry, agriculture, and the food industry into high-quality nutrients without burdening natural resources. To increase their share, these new ingredients must meet the requirements of being available in large quantities, providing a predictable supply throughout the year, and offering competitive prices for functional use (Albrektsen et al. 2022). Commercial production of microbial ingredients is under development and several start-up companies have been established, although current production volumes are not known (Albrektsen et al. 2022).

Future challenges for insect farming for feed production include competition for organic waste with other new industries in the circular bioeconomy, which could increase insect feed prices (Albrektsen et al. 2022). Whereby with regard to the use of products from various streams in the feed, special attention must always be paid to the composition of the insects produced (Montalbán et al. 2022, Riekkinen et al. 2022). The introduction of regulations on the use of insects as a food source means that the market will begin to consume more insect meal, which, in turn, will affect the projected growth volume for insect meal for feed (Albrektsen et al. 2022).

Based on the current state of knowledge, the authors think that relevant quantities of new by-products for animal nutrition based on Novel Foods are questionable to date. The extent to which nutritional trends based on Novel Foods directly displace relevant quantities of classic foods of animal origin is still difficult to assess at present. With regard to strong nutritional trends, however, another issue is certainly relevant, namely the increased production of by-products in the course of the production of vegetarian and vegan foods and their use in animal nutrition. In this context, new processing technologies and refining methods to ensure the commercial production of nutritionally healthy and efficient feed ingredients are of high importance (Albrektsen et al. 2022).

Overall, the essential goal will be to reduce the share of crops used directly as animal feed. According to a recent study (Detzel et al. 2022), in 2022, 67% of cereals, 58% of pulses and 93% of soybeans in the EU-28 were used for animal feed. Here, however, it is paramount to explore all dimensions of sustainability and the trade-offs that novel ingredients may entail, including their impact on the environment, biodiversity, ecosystem conservation, greenhouse gas emission reductions, and social and economic outcomes (Albrektsen et al. 2022).

Future Perspectives

A “safe-by-design” approach, where food and feed safety considerations are taken into account at the design stage of new products, should be applied to reduce compliance monitoring costs. The balance between “environmental, economic and safety aspects” should be included in such assessments (Van Raamsdonk et al. 2023).

If it will become technically feasible to use Novel Feeds in the future, evaluation standards for sustainability assessment must also be available. This is crucial for sustainability improvement approaches, because if the feed itself does not have an internationally accepted "footprint assessment", then no benefits with regard to sustainability enhancement can be displayed, both validly and internationally recognized. Otherwise, the use of the Novel Feed may become redundant.

Novel Feeds could thus become relevant only after a rather a bit longer period of sustainability assessment, as impacts can only be shown after period of assessment. Alternatively, preliminary derivations from non-novel components will have to be used.

The science related to New Feeds is exciting, but the extent to which it can become relevant depends largely on social, legal and economic linkages and regulations, as well as other players (e.g. food retailers).

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Abstracts

1.

Influence of substituting peptide-bound with free amino acids on growth and nitrogen metabolism of broiler chickens depending on asparagine and glutamine supply

Einfluss des Austausches von peptidgebundenen gegen freie Aminosäuren auf Wachstum und Stickstoff-Stoffwechsel bei Broilern in Abhängigkeit von der Asparagin- und Glutamin-Versorgung

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Reducing dietary crude protein (CP) is a strategy to reduce nitrogen (N) excretion by broilers. Free amino acids (FAA) allow to lower CP in feed considerably without an amino acid (AA) deficit. Absorption rates between peptide-bound and FAA differ, which may lead to an imbalanced pattern of absorbed AA. This interpretation led to the suggestion an upper limit of FAA inclusion may exist above which growth is impaired [1]. There is only one study on broilers that replaced peptide-bound AA with FAA. In this study, AA contained in soy protein isolate (SPI), which accounted for 41% of dietary CP, were substituted with the same amount of FAA. The growth of birds fed with the FAA diet was reduced, but nitrogen utilisation efficiency (NUE) was unaffected [2]. However, AA digestibility of SPI and FAA differs and asparagine (Asn) and glutamine (Gln) may have limited growth in that study. The present study investigated consequences of an incremental substitution of digestible AA in SPI with FAA depending on the Asn and Gln supply.

Methods: Nine diets mainly containing maize, maize starch and casein were prepared. A diet with 191 g CP/kg dry matter contained 80 g SPI/kg (~38% of dietary CP). In the other diets, 25, 50, 75 and 100% of the digestible AA contained in SPI were substituted with FAA. Aspartic acid (Asp) and glutamic acid (Glu) in SPI were substituted with Asp and Glu, or with 50/50 mixes of Asp/Asn and Glu/Gln, respectively. Precaecal AA digestibility of SPI was determined before. 630 broilers were raised in floor pens on litter and fed with a commercial starter. The birds were distributed to 63 metabolism units of 10 birds each on day (d) 6. Each diet was tested in 7 replicates. The experimental diets were fed from d 7–21. Total excreta were collected twice daily on d 11–14 and 18–21 to determine N accretion and NUE. Effects of diets were statistically analysed by one-way ANOVA.

Results: Average daily gain (ADG), average daily feed intake (ADFI) and gain:feed (G:F) from d 7–21 was unaffected by 25% substitution and at 60 g/d, 67 g/d and 0.90 g/g, respectively, and decreased continuously to 52 g/d, 59 g/d, and 0.88 g/g, respectively, at 100% substitution when Asn and Gln were not added ($P<0.001$). When Asn and Gln were added, ADG, ADFI and G:F were unaffected up to 50% substitution and decreased continuously to 49 g/d, 56 g/d and 0.87 g/g, respectively at 100% substitution ($P<0.001$). N accretion was unaffected by 25% substitution at 1.31 and 2.12 g/d on d 11–14 and 18–21, respectively, and decreased continuously to 1.16 and 1.86 g/d, respectively, at 100% substitution when Asn and Gln were not added ($P\leq0.004$). When Asn and Gln were added, N accretion on d 11–14 and 18–21 was unaffected up to 50% substitution and decreased continuously to 1.11 g/d and 1.78 g/d, respectively, at 100% substitution ($P<0.001$). The NUE on d 11–14 decreased up to 50% substitution from 76% to 74% ($P=0.002$) and remained at this level at higher substitution when Asn and Gln were added. When Asn and Gln were not added, NUE was unaffected up to 50% substitution at a level of 75% ($P=0.102$) and then increased to 77% at 100% substitution ($P<0.001$). On d 18–21, NUE decreased up to 50% substitution from 75% to 72% irrespective of whether Asn and Gln were added ($P\leq0.029$). The NUE increased at higher AA substitution to 76% and 74% at 100% substitution in diets without and with Asn and Gln addition, respectively ($P\leq0.045$).

Conclusions: There was a maximum of FAA inclusion without effects on growth and N accretion and this maximum depended on whether free Asn and Gln was supplied. Supplying free Asn and Gln increased the maximum of AA substitution without reduced growth and N accretion from 10% to 19% of dietary CP. This suggests that Asn, Gln or both were growth-limiting. The decrease and increase of NUE with increasing AA substitution was influenced by decreasing proportions of non-AA-N and may be explained by reabsorption of urinary N and lower protein turnover.

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2.

Effect of replacement of casein with *Hermetia illucens* larvae meal as dietary protein source on lipid metabolism in obese Zucker rats

Wirkung des Austauschs von Casein durch Hermetia illucens-Larvenmehl als diätetische Proteinquelle auf den Lipidstoffwechsel von fettleibigen Zuckerratten

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Insect larvae meal has been increasingly recognized as a sustainable dietary protein source, because it can be efficiently produced from suitable edible insect species, such as *Tenebrio molitor* (TM) and *Hermetia illucens* (HI), with low environmental impact. Protein-rich insect meal might be also interesting due to various health-related effects, such as antisteatotic [1], hypocholesterolemic [1], antihypertensive [2] and antiadipogenic [3] actions, which have been observed in different rodent models. Since all of these health-related effects have been observed with the use of *Tenebrio molitor* (TM) larvae meal, the question arises if such effects can be also observed with the use of HI larvae meal. This, however, is currently unknown from the available literature. In order to close this gap of knowledge, the present study tested the hypothesis that dietary HI larvae meal attenuates the development of liver steatosis and hyperlipidemia in the obese Zucker rat.

Methods: A 4-week feeding trial with male, 10-wk-old obese Zucker rats (n = 30) and male, 10-wk-old lean Zucker rats (n = 10) was performed. The obese rats were randomly divided into three obese groups (group O-C, group O-HI25, group O-HI50) of 10 rats each. The lean rats served as a lean control group (L-C). Groups L-C and O-C were fed a control diet with 20% casein as protein source, whereas in groups O-HI25 and O-HI50 25% and 50%, respectively, of the casein was replaced isonitrogenously by HI larvae meal. The concentrations of the major fatty acids were similar in the three diets due to adjustment of fatty acid composition by using individual amounts of different dietary fats. After killing the animals, blood plasma and liver were collected. Concentrations of triglycerides and cholesterol in plasma and liver lipid extracts were determined by enzymatic test kits. Fatty acid composition of hepatic total lipids was analyzed by gas chromatography-flame ionization detection. Hepatic lipid accumulation was evaluated by Oil Red O-staining. Hepatic mRNA levels of lipogenic genes were determined by qPCR. The activity of lipogenic enzymes in liver cytosolic fractions was determined using a commercial test kit (glucose-6-phosphate dehydrogenase (G6PD)) and by monitoring NADP+ reduction during incubation in the presence of malate and NADP+ (malic enzyme (ME)). Groups L-C vs. O-C were statistically compared by t-test and Mann-Whitney U test for normally and non-normally distributed data, respectively. 1-way ANOVA and Kruskal-Wallis test were used for analysis of normally and non-normally distributed data, respectively, within the obese groups.

Results: Growth performance was higher in group O-C than in group L-C ($P<0.05$), but did not differ among the obese groups. Oil red O-staining of liver sections revealed an excessive lipid accumulation in the liver of group O-C, whereas liver lipid accumulation in group O-HI25 and O-HI50 was markedly reduced compared to group O-C. No lipid accumulation was found in the stained liver sections of group L-C. Absolute concentrations of C14:0, C16:0, C16:1, C18:0, C18:1 and total fatty acids in the liver were markedly higher in group O-C than in group L-C, but lower in group O-HI100 than in group O-C ($P<0.05$). Hepatic concentrations of triglycerides and cholesterol, plasma concentration of cholesterol, hepatic mRNA levels of the lipogenic genes G6pd, Me1, Acly (ATP citrate lyase), Acaca (acetyl-CoA carboxylase alpha), Fasn (fatty acid synthase) and Scd1 (stearoyl-CoA desaturase 1) and hepatic activities of G6PD and ME were higher in group O-C than in group L-C ($P<0.05$), but lower in group O-HI100 than in group O-C ($P<0.05$).

Conclusions: Isonitrogenous replacement of casein by HI larvae meal as a dietary protein source exerts favorable metabolic effects in a rat model of liver steatosis and hyperlipidemia. This suggests that HI larvae meal, like TM larvae meal, might be a suitable protein source to induce relevant health-related effects.

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3.

Glutamine metabolism in low birthweight suckling piglets

Glutamin-Stoffwechsel bei Saugferkeln mit niedrigem Geburtsgewicht

*De Leonardis D., Sciascia Q. L., Görs S., Vernunft A., Metges C. C. – Dummerstorf

Low birthweight (L) piglets have impaired growth and compromised gastrointestinal tract (GIT) development compared to their normal birthweight (N) littermates [1]. Glutamine (Gln) is a major metabolic substrate for enterocytes of the GIT and orally supplemented Gln has been reported to improve suckling piglet bodyweight (BW) [2]. However, it is not known if the oxidative fate of Gln differs between L and N piglets. Therefore, this study aimed to follow $^{13}C_5$ Gln oxidation, administered orally to L and N suckling piglets.

Methods: Trial 1: To investigate if breath $^{13}CO_2$ enrichment was similar to red blood cells (RBC), 2 male piglets (12 days of age (d)) were implanted with a jugular catheter. At 14 d an oral dose of $^{13}C_5$ Gln (10 mg/kg BW) and unlabelled Gln (333 mg/kg BW) dissolved in water was given. Blood and breath samples were collected at -15 (basal), 30, 60, 90, 120, 150, 180, 210, 240 and 300 min after tracer administration. Trial 2: At birth (1 d), 16 male German Landrace piglets from parity 2–9 sows with litter sizes of 12–20 piglets and at least one L (0.8–1.2 kg) and N (1.5–1.9 kg) littermate, were selected. At 24 hours (h) post farrowing, litter sizes were standardized to 14 piglets, and experimental animals were assigned to either a Gln (1 g/kg BW/d) or a water (W) supplementation groups (L-Gln, L-W, N-Gln, N-W; n = 4 per group). Piglets were orally supplemented with 33% of their daily dose of Gln solved in water or 2 ml of water at 3 different time points (7, 12, and 17h), from 2 to 16 d. Bodyweight was recorded daily, crump-rump length (CRL) and abdominal circumference (ABC) were recorded at 1, 2 and 8 d, and at age 14 d, all piglets were given $^{13}C_5$ Gln and blood was sampled as described for Trial 1. Gas chromatography–mass spectrometry and isotope-ratio mass spectrometry were used to measure plasma $^{13}C_5$ Gln enrichment, and breath and RBC $^{13}CO_2$ enrichment, respectively. Area under the enrichment-time-curve (AUC), maximum enrichment (Emax) and time to maximum enrichment (tmax) were calculated by curve fitting (TableCurve 2Dv5.01). Trial 1 data was analyzed in Excel, whilst Trial 2 data was analysed using an ANOVA procedure with repeated measures where appropriate (SAS).

Results: Trial 1: Breath and RBC $^{13}CO_2$ enrichment correlated well (r = 0.99, ERBC = 0.9023 Ebreath), thus RBC were used for Trial 2 $^{13}CO_2$ enrichment measurements. Trial 2: L piglets were lighter (2.14 vs. 3.14 kg, $p<0.001$), smaller (CRL, 25.2 vs. 28.8 cm; ABC, 25.2 vs. 28.5 cm, $p<0.05$), and gained less BW between birth and 14 d (230 vs. 343 g/d, $p<0.001$) than N littermates. At 8 d, L-Gln piglets were smaller (ABC, 28.5 vs. 31.5 cm, $p<0.05$) and shorter (CRL; 27.9 vs. 30.7 cm, $p<0.05$) than L-W littermates. The tmax of plasma $^{13}C_5$ Gln enrichment was shorter (28.4 vs. 45.0 min, $p<0.05$) in L-Gln than L-W. No differences in plasma $^{13}C_5$ Gln AUC (157 ± 19.9 mole % excess (MPE) · min) or Emax (1.0 ± 0.1 MPE), and RBC $^{13}CO_2$ enrichment AUC (16.5 ± 2.0 atom % excess (APE) · min), Emax (0.1 ± 0.01 APE) and tmax (64 ± 6.9 min) between L-Gln and L-W piglets, respectively, were observed ($p>0.05$). In addition, $^{13}C_5$ Gln plasma enrichment and RBC $^{13}CO_2$ appearance in N piglets were not different between Gln and water supplementation groups and was comparable to L piglets.

Conclusions: These results suggest that Gln supplementation does not improve growth of the L piglets used in this study, but appears to reduce the time of maximum plasma Gln $^{13}C_5$ enrichment. The reduced time of maximum plasma Gln $^{13}C_5$ enrichment suggests that in L piglets Gln supplementation increases GIT Gln absorption or changes the metabolic kinetics. We hypothesize that this could be due to increased Gln transporter number or function, a possibility that should be investigated.

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4.

Influence of oral glutamine supplementation on the abundance of gene expression and metabolic pathways in digesta of jejunum in neonatal piglets

Einfluss einer oralen Glutaminergänzung auf die Genexpression und Stoffwechselwege in der Digesta des Jejunums von neonatalen Ferkeln

*Schulze Holthausen J., Sciascia Q. L., Schregel J., Li Z., Andreotti S., Vahjen W., Metges C. C., Zentek J. – Berlin / Dummerstorf

The non-essential amino acid glutamine (Gln) has been shown to improve growth and immunological functions in pigs after weaning [1]. Low birthweight piglets (LBW) typically face a higher risk of mortality before weaning, often due to impaired gastrointestinal function [2]. *In vitro* studies have shown, that Gln is used by small intestinal bacteria and also affects the utilization pattern of other amino acids [3]. In a previous study we observed, that Gln affects the main abundant Lactobacillus spp. in jejunal digesta. The aim of this study was to investigate changes in bacterial gene expression and metabolic pathways in jejunal digesta of LBW and normal birthweight (NBW) piglets under the influence of Gln or alanine (Ala) supplementation.

Methods: For the trialLBW (0.8 - 1.2 kg) and NBW (1.4 - 1.8 kg) male littermates born to gilts were paired at birth (day (d) 0). The piglets received an oral supplementation of either 1 g Gln or isonitrogenous Ala (1.22 g/kg body-weight) until 12 d. Thus, four different groups were studied: LBW+Gln; NBW+Gln; LBW+Ala; NBW+Ala (n = 5/ age group). Piglets suckled throughout the study. Animals were euthanized at 5 and 12 d of life (n =40) and jejunal digesta was snap frozen in liquid nitrogen, and stored at -80°C for subsequent analysis. DNA was extracted from the digesta and full metagenomic sequences were analysed. Statistical analyses were performed using the R package MaAslin2. Using this package differences in gene abundance and metabolic pathways between supplementation, birthweight and age groups were considered statistically significant at $q < 0.05$. In addition, significant associations of the factor's supplementation, birthweight, and age with the metabolic pathways were determined.

Results: In total32156 genes were detected in jejunal digesta of suckling piglets. Of the detected genes in the digesta of jejunum, 22 genes showed a significant change in abundance ($q < 0.05$) affected by Gln supplementation. Out of all the detected genes, 18 known genes were higher abundant in Gln compared to Ala supplemented piglets. Most of the upregulated genes of jejunal digesta influenced by Gln supplementation were related to Lactobacillus spp., specifically L. limosilactobacillus mucosae. The molecular function of these higher abundant genes was mainly associated with DNA, rRNA, metal ion, ATP and NAD or NADP binding, hydrolase activity and amino acid transporter activity. Significantly higher abundant genes were involved in the biological processes of DNA replication and repair, translation, transcription as well as in glucose, carbohydrate and nitrogen metabolism. There were no significant changes in gene abundance influenced by birthweight. Age showed a total of 4366 significantly changed genes in jejunal digesta, 2965 with a known and 1401 without a specific gene name ($q < 0.05$). Of the 2965 known genes, 2119 genes had a higher abundance at 12 d compared to 5 d and 846 genes were lower abundant at 12 d compared to 5 d. A total of 597 different metabolic pathways were detected in jejunal digesta. Gln supplementation showed a negative association to the pyruvate fermentation to acetate and lactate. LBW had a positive association with the dezapurine biosynthesis pathway, superpathway of fermentation of Chlamydomonas reinhardtii and phospholipid biosynthesis in bacteria. Age had 17 positive associations with different pathways majorly related to DNA function. Negatively associated with age were 7 pathways mainly related to heterolactic fermentation and pyruvate fermentation to acetate and lactate.

Conclusions: Overall, the results demonstrate a potential of Gln supplementation to influence gene expression of bacteria and to modulate some metabolic pathways in the digesta of male suckling piglets. Birthweight did not influence the gene abundance in digesta and showed little effects on metabolic pathways, whereas age had the major influence on the gene abundance and metabolic pathways in digesta of male suckling piglets.

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5.

Evaluating the isoleucine requirement in post-weaning piglets

Untersuchungen zum Bedarf von Isoleucin bei Absatzferkeln

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Low crude protein (CP) diets are an important part in the discussion about resource-efficient animal production. To avoid over- or undersupply of essential amino acids in piglet diets, it is important to know the animals exact requirements. Isoleucine (Ile) can be regarded as next liming amino acid after valine [1] and its requirement needs to be adjusted to the genetically improved growth potential of pigs. Additionally, studies on the Ile requirement in post weaning pigs show variable results. Therefore, the present study investigated the Ile requirement in post-weaning piglets.

Methods: A total of 384 post-weaning piglets were randomly allocated to one of six dietary treatments. The piglets (64 piglets per treatment, 8 pens x 8 piglets) received a diet with incremental levels of dietary standardized ileal digestible (SID) Ile, by including stepwise free L-Ile in the range of deficient till above the assumed requirement value. The basal diet (T1) consisted mainly of corn, wheat, barley, potato protein, hemoglobin powder and soybean meal (CP: 15 %; Net energy: 10.46 MJ/kg; SID Lysine: 12.0 g/kg) and was deficient in Ile (4.40 g SID Ile/kg of the diet). In the treatments T2 to T6, L-Ile (BESTAMINOTM, CJ BIO) was included in the basal diet at the expense of maize starch to reach SID Ile contents of 4.95, 5.50, 6.05, 6.60, and 7.15 g/kg, respectively. Feed intake (FI), body weight gain (BWG) and feed conversion ratio (FCR) were measured after 4 weeks of feeding period, starting 6 days post-weaning. Regression analysis with a broken line model, a quadratic model and an exponential model was applied to derive requirement values for Ile. Based on the R square value of the models, the exponential model (97.5% of the maximum response) was retained as the best fitting model for the observed responses.

Results: The piglets fed the basal diet, had significantly lower BWG, FI, and FCR ($P<0.05$) than animals fed with Ile supplemented diets suggesting that the basal diet was truly deficient in Ile. FI and ADG showed a dose dependent response towards the dietary L-Ile level. The requirement values for Ile, based on regression analysis with the exponential model were estimated at 6.2 and 6.1 g SID Ile/kg for FI and BWG, respectively. No requirement value could be derived based on the FCR, as no clear dose response relationship was observed.

Conclusions: Inclusion of free L-Ile in an Ile deficient diet led to a gradual increase in growth performance. The isoleucine requirement in piglets during the post-weaning period amounts 6.1 g SID Ile/kg of diet, equivalent to 51% relative to SID lysine.

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6.

Effect of reduced dietary nitrogen (N) and phosphorus (P) on performance, nutrient digestibility and N-retention of modern fattening pigs

Effekt einer Stickstoff- (N) und Phosphor- (P) reduzierten Fütterung auf Leistung, Nährstoffverdaulichkeit und N-Retention moderner Mastschweine

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The amendment of 2017 to the German Fertilizer Ordinance severely restricted the environmental output of N and P, including that derived from pig's manure. Therefore, feeding diets drastically reduced in N and P are recommended to farmers as one possible approach [1]. However, currently used databases on body accretion are ~20 years old (N retention: 25.6 g/kg BW gain) [1], potentially resulting in inaccurate calculations. This poses the question to what extent body accretion has changed in modern fattening pigs and whether drastically reduced N/P levels may affect porcine nutrient utilization.

Methods: Two diets were formulated, a N/P-reduced diet (NPred) [1] and a control (CON) meeting GfE recommendations [2]. Diets were based on barley, wheat and soybean meal with no exogenous phytases added and applied in a 3-phased dietary regimen (starter, grower and finisher phase) as meal. Dietary crude protein (g/kg feed) for CON/NPred was calculated at 175/150 for starter, 160/130 for grower and 145/120 for finisher phase, respectively and phosphorus (g/kg feed) set at 4.5/4.0 (CON/NPred), independent of feeding phase. Eight barrows (BHZP) were surgically fitted with a simple T-cannula at the terminal ileum (32.7 ± 2.7 kg BW) and allotted to NPred or CON (n=4/diet). Pigs were fed ad libitum with each dietary phase lasting 3 weeks. The last week of each phase consisted of five consecutive days of quantitative faeces and urine collection. Feed intake was measured daily per pig and pigs were weighed before and after each collection period. Nutrient analysis was performed in feed, faeces and in urine and apparent total tract digestibility (ATTD) as well as N-retention were calculated. Statistical analysis was performed with phase, diet and their interactions as fixed factors (phase as repeated measurement) and data were deemed significant at $p<0.05$ (Tukey-Kramer post-hoc test). Minerals are currently analyzed and thus not subject of this abstract.

Results: Live weight gain (LWG), daily feed intake (DFI) and feed-to-gain ratio (F:G) were significantly affected by phase only. In the starter phase, LWG was significantly lower (871 g) than in grower (1069 g) and finisher phase (1005 g). DFI increased linearly from starter (3.2 kg), grower (3.5 kg) to finisher phase (3.7 kg). F:G (kg feed/kg LWG) ranged between 3.7 (starter, finisher) and 3.3 (grower phase). Diet had no significant influence on performance. In general, ATTD of nutrients was significantly different due to feeding phase as well as diet, but showed no interaction between both factors. ATTD of organic matter (OM) and lipid (CP) was significantly higher in NPred-fed pigs (OM %: 86.6 vs. 88.0; CL %: 72.2 vs. 75.2 for CON vs. NPred). The same was observed for ATTD of protein, albeit as a trend (CON vs. NPred: 81.0 vs. 82.7%, $p_{diet}=0.07$). ME was significantly lower in CON compared to NPred-fed pigs (15.2 vs. 15.4 MJ/kg DM). In contrast, ATTD of crude fiber, NDF, ADF and NfE were not significantly influenced by diet, but by feeding phase only. Excreted faecal N was significantly lower in NPred-fed pigs compared to CON (CON vs. NPred: 15.6 vs. 12.7 g/d, $p_{diet}=0.008$). Furthermore, faecal N excretion ($p_{phase}=0.002$) was significantly higher in grower phase (16.9 g/d) compared to starter phase (11.9 g/d) and finisher phase (13.7 g/d). Urinary N excretion in pigs fed NPred represented only 62% of CON-fed counterparts (CON vs. NPred: 16.8 vs. 10.5 g/d, pdiet=0.001). N retention related to metabolic body weight (g/kg $BW^{0.67}$) significantly declined from starter to finisher phase ($p_{phase}<0.001$) with 2.8 in starter, 2.3 in grower and 1.9 g/kg $BW^{0.67}$ in finisher phase, whereas diet had no significant effect.

Conclusions: Our balance data show that body accretion capacity in today's high-yielding fattening pigs is markedly enhanced, in particular with respect to nitrogen [1]. Furthermore, feeding a N/P-reduced diet resulted in improved nutrient digestibility and equal performance, demonstrating an improved nutrient efficiency.

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7.

Evaluation of purine derivatives and other nitrogen compounds in urine as markers for an optimal nitrogen supply to pigs

Bewertung von Purinderivaten und weiteren Stickstoffverbindungen im Harn als Indikatoren für eine optimierte Stickstoffversorgung von Schweinen

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Nitrogen (N) in the form of amino acids is an essential nutrient for animals and, at the same time, nitrogenous metabolites form a major pollutant from agriculture. In ruminants, urinary N compounds have already been used extensively to estimate the origin (microbial, dietary or endogenous) of the N excreted. Purines and other N compounds in urine have not received the same attention in pigs, although the information could be used to better understand N losses and to distinguish between inevitable and regulatory N losses. Therefore, urine samples from a balance trial on growing pigs were used to quantify purine derivatives and other N compounds.

Methods: Two balance trials were conducted, each with 12 castrated male crossbred pigs. Eight different compound feeds, consisting of 70% cereal grains, either wheat (W) or rye (R), and 30% protein supplement, either soybean (SBM) or rapeseed (RSM) meal, were tested with and without added phytase. The rations were not isoenergetic or isonitrogenous as they were designed to reach a targed concentration of digestible P (dP) of 2 g/kg. Pigs [average initial body weight (SD): 34.2 kg (5.8 kg); 28.2 kg (6.0 kg)] were assigned to duplicate 3 x 3 Latin squares and randomly allotted to metabolism crates. The animals were fed twice daily at 07.30 h and 15.30 h and consumed their feed in amounts corresponding to approx. 2.5 multiples of their ME requirement for maintenance. After an adaption period of 7 days, faeces and urine were collected quantitatively over a 5-day period. To avoid volatile N losses at sampling time urine was acidified with sulfuric acid to ensure a pH of ≤ 3.0. Daily aliquots of the urine were stored frozen until analysis. Urine samples of each pig and collection period were pooled. Total N in feeds and urine samples was analysed using the Kjeldahl method. Additionally, the following urinary N compounds were analysed: urea, ammonia, purine derivatives (i.e., allantoine (AL), uric acid, hypoxanthine (HX), xanthine (X)), hippuric acid and creatinine. The MIXED procedure of SAS (version 9.4; SAS, Inst., Inc., Cary, NC, USA) was used to estimate effects of grain source, protein supplement and phytase supplementation, and their interactions, on urinary excretion of N compounds. .

Results: The crude protein (N ·6.25) concentration of the rations varied between 193 and 223 g/kg DM. The total N excreted via urine ranged between 10.4 and 18.3 g N/day. Pigs fed rations with W, SBM and phytase unsupplemented, excreted significantly more total N via urine than those fed R, RSM and supplemented with phytase, which is related to the different N intakes. Urea accounted for 74 to 84% of total urinary N with the same differences observed for N. The calculated urinary non-urea N excretion was affected by phytase supplementation, which was likely caused by differences between unsupplemented and phytase supplemented rations in N concentration. Excretion of AL was significantly higher in urine of pigs fed rations containing rye. In the urine of pigs fed RSM, concentrations of X and HA were significantly higher, whereas HX was significantly lower. However, both X and HX were excreted in very small amounts (X: 4.56-8.94 mg N/day; HX: 18.0-24.8 mg N/day). The unidentified N fractions, i.e. the proportion of total N which could not be assigned to one of the analysed N fractions, accounted for 49.1 to 59.7% of the urinary non-urea N in the present study.

Conclusions: About 80% of the N excreted in urine of pigs is urea, which is supported by the present results. Urea forms the major source of ammonia release from manure and thus may negatively impact on pig health and the environment [1]. Therefore, it appears rewarding to investigate further if urinary non-urea N can serve as an indicator for unavoidable N losses in pigs and to which extent N supply can be reduced to lower urinary urea-N excretion and the resulting N emission in the form of ammonia.

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8.

A simple laboratory method to estimate standardised precaecally digestible amino acids for pigs

Eine einfache Labormethode zur Schätzung der standardisiert praecaecal verdaulichen Aminosäuren beim Schwein

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The adequate protein supply to pigs to ensure performance and animal health and to reduce nitrogen losses, which are harmful to the animal and to the environment, can be achieved by a precise protein evaluation of the feed. Protein evaluation of pig feeds is based on the standardised precaecally digestible crude protein (spcdCP) [1]. The *in vivo* spcdCP values are determined using an invasive method using ileal cannulae. *In vitro*, spcdCP has been determined using a time-consuming multi-enzyme method [2]. Therefore, the objective was to develop a rapid and cost-effective laboratory method for the estimation of spcdCP and spcd amino acids (spcdAA) in pig feeds. Analogous to the protein fractionation of ruminant feeds [3], spcdCP and spcdAA were determined based on neutral (NDICP) or acid detergent insoluble crude protein (ADICP) and AA (NDIAA/ADIAA), respectively. Based on analysed fractions, the ND or AD soluble CP (NDSCP/ADSCP) or AA (NDSAA/ADSAA) fractions were estimated and used to estimate *in vivo* values. Below, the focus is laid on scpAA.

Methods: The laboratory method is based on the knowledge that pigs degrade and ferment cell-wall material only in the large intestine. This means that NDIAA and ADIAA are virtually indigestible in the small intestine. In contrast, the NDSAA and ADSAA fractions are available to the animal in the small intestine. This results in the following relationships:

$NDSAA = AA_{feed} - NDIAA$

$ADSAA = AA_{feed} - ADIAA$

A unique, large sample pool of more than 80 straight feedingstuffs (protein sources, e.g., differently heat-treated rapeseed and soybean products, fava beans, lupines, field peas, and cereal grains such as wheat, barley, triticale and rye) was available for which *in vivo* spcdCP and spcdAA values were determined in pigs. Isolation of NDIAA and ADIAA were carried out using established methods for fibre analyses of feeds. Amino acid concentrations in the detergent residues (NDIAA, ADIAA) were determined by HPLC. The concentrations of NDSAA and ADSAA were calculated by difference, using the above relationship. These values were then used to estimate *in vivo* spcdAA concentrations (g/kg dry matter [DM]). Linear regression analysis was performed on this data and an ANOVA and subsequent Tukey test were performed to determine the differences between the cereal grain types.

Results: In general, laboratory values (NDSAA or ADSAA, x) showed a good performance to estimate *in vivo* pcdAA (y) . Here examples for lysine, methionine and threonine are presented. Cereal grains were divided into two groups: wheat/triticale and rye/barley.

Lysine:
Wheat/Triticale: $y = 0.8709\ x - 0.1299$ $R^2 = 0.938$
Barley/Rye: $y = 0.5318\ x + 0.6784$ $R^2 = 0.681$
Protein supplements: $y = 0.9017\ x - 3.8303$ $R^2 = 0.995$

Methionine:
Wheat/Triticale: $y = 0.8661\ x + 0.0951$ $R^2 = 0.963$
Barley/Rye: $y = 0.7554\ x + 0.1508$ $R^2 = 0.975$
Protein supplements: $y = 0.8997\ x - 0.0855$ $R^2 = 0.999$

Threonine:
Wheat/Triticale: $y = 0.7272\ x + 0.4809$ $R^2 = 0.865$
Barley/Rye: $y = 0.6956\ x + 0.2023$ $R^2 = 0.902$
Protein supplements: $y = 0.8236\ x - 1.2778$ $R^2 = 0.996$

Conclusions: Determination of NDIAA and ADIAA can be performed as a routine analysis for AA evaluation. Therefore, the rapid and cost-effective laboratory method is an alternative to the *in vitro* multienzyme method to estimate spcdAA values from routinely available chemical feedstuff characteristics.

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9.

Effects of varying ruminal N-balance (RNB) on performance and N excretion of dairy cows in late lactation

Zum Einfluss einer variierenden ruminalen N-Bilanz (RNB) auf Leistungskriterien und die N-Ausscheidung bei Milchkühen in der Spätlaktation

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Reduction of dietary crude protein (CP) concentration at a contemporary adequate supply with utilizable crude protein at the duodenum (uCP) and amino acids is an effective way to reduce nitrogen excretion of dairy cows. In practice a reduced dietary protein supply can especially be achieved for cows in later lactation by introducing phased feeding systems. However, most studies on protein and amino acid nutrition were conducted in high yielding dairy cows at the start of lactation and information for late lactation is relatively scarce. For this reason, the present study aimed to investigate the effects of varying dietary ruminal nitrogen balance on performance of dairy cows in late lactation.

Methods: The feeding trial started with 48 dairy cows and lasted for 10 months. Cows were allocated to two groups (RNB+ and RNB-) according to breed, milk yield, lactation number, and stage of lactation. Dry off cows were replaced by other cows, so that a total of 80 Simmental and 28 brown Swiss cows was involved. At the start of the trial, cows were on average 223 days in milk. The cows had ad libitum access to partial mixed rations (PMR) based on maize and grass silages, hay and concentrates. In group RNB+ the main concentrates included in PMR were maize and extracted rapeseed meal. For group RNB- extracted rapeseed meal was partly replaced by maize and barley in order to achieve lower RNB at comparable energy and uCP concentration of diets. Additional concentrates were offered according to actual milk yield (0.4 kg/kg milk). Individual feed intake and milk yield was automatically recorded daily and milk samples for analysis of milk composition were taken in weekly intervalls. Spot samples of urine and faeces were collected during five days from a subset of 10 cows per treatment. Daily excretion of urine was calculated from potassium intake, potassium excretion with milk and urinary potassium concentration, according to the method given by Bannink et. al. (1999) [1]. Daily excretion of faeces was calculated from faecal organic matter. Performance data were evaluated by a mixed model using SAS. Nitrogen excretion data were evaluated by one-factorial ANOVA using SAS. Results are presented as lsmeans±standard error.

Results: Concentration of uCP and RNB in the diets for groups RNB+ (148 and 0.8 g/kg DM) and RNB- (143 and -1.3 g/kg DM) were lower than calculated. Daily intake of dry matter (19.4 vs. 20.6 kg, ±0.3), CP (2581 vs. 3151 g, ±63), and uCP (2787 vs. 3053 g, ±57) in group RNB- was lower ($p<0.05$) than in group RNB+. Daily milk and energy-corrected milk yield were numerically reduced by about 2 kg per day in group RNB- ($p=0.112$ and $p=0.071$). Milk protein yield and milk urea concentration was lower ($p<0.05$) in group RNB- compared to group RNB+. Daily excretion of nitrogen with urine was lower in group RNB- than in group RNB+ (115 and 160 g, ±8, $p<0.05$), whereas nitrogen excretion with faeces did not differ between groups (145 and 167 g/day, ±10). There was also no difference in N emissions per kg edible protein (0.32 kg N/kg edible protein in group RNB- and 0.36 kg N/kg edible protein in group RNB+, $p=0.387$). The higher N excretion via urine in group RNB+ was due to significantly higher urinary urea levels. There was no effect of dietary treatment on body weight, body condition score, and backfat thickness.

Conclusions: A negative RNB of -1.7 g/kg DM in late lactation led to a reduced feed intake and subsequently to a reduction of milk yield of about 2 kg/d under the condition of this trial. Even if such a nitrogen reduced feeding regimen reduces N emission, it is not recommended for practical purpose to date because it may negatively impact cows' performance.

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10.

A study on the possibility of taurine synthesis in *in vitro* rumen fermentation

Eine Studie zur Möglichkeit der Taurinsynthese in der in vitro Pansenfermentation

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Taurine is commonly referred to as a semi-essential amino acid in new born mammals [1]. Taurine can be synthesized in animal liver using methionine (Met) as the main precursor, but the amount of taurine synthesized in animal body can only meet 30-40% of animal's need. It was reported that Corynebacterium glutamicum synthesized taurine in *in vitro* incubation [2]. Since ruminal microbiota is diverse and complex, we hypothesized that some ruminal bacteria could synthesize taurine.

Methods: The *in vitro* rumen fermentation technique of Zhao and Lebzien (2000) was used for incubation [3]. Two adult beef cattle fitted with rumen cannulas and fed with a total mixed ration composed of corn silage and concentrate mixture (roughage/concentrates = 55/45, dry matter basis) were used as the donors of rumen fluid. Twenty centrifuge tubes with 50 mL of calibrated volume were used as incubation vessels. Each tube contained 0.4000 g feed mixture (roughage/concentrates = 50/50, air-dry basis) and 8.0 mg (2% air-dry feed) free Met (purity 99%). Four tubes were taken at 2, 4, 8, 12 and 24 h of incubation, respectively, and kept frozen at -20°C. The microbial mass of incubation liquid was broken on a sonicator (XL-2000; Aoran Science and Technology Co., Ltd., Shanghai, China). The Met and taurine in incubation liquid were analysed using a liquid chromatography-tandem mass spectrometry (TSQ Quantum Access MAX; Ultimate 3000; Thermo Fisher Scientific, Inc., USA). The ruminal pH was measured using a portable pH meter. The ammonia nitrogen (NH_3-N) and microbial crude protein (MCP) were analyzed on a spectrophotometer (UV-1801; Beijing Rayleigh Analytical Instrument Co. Ltd., Beijing, China) using colorimetric methods. The linear effects of incubation time on different parameters were evaluated using SPSS 17.0. Effects were considered significant at $P \leq 0.05$, with tendency at $0.05 < P \leq 0.10$.

Results: The pH values of the incubation liquids at different time points was 6.5-7.0. The Met concentrations of incubation liquids at 2, 4, 8, 12 and 24 h of incubation were 4.72 ± 0.134, 3.46 ± 0.284, 3.06 ± 0.193, 1.71 ± 0.146 and 0.11 ± 0.005 ppm, respectively, which linearly decreased with incubation time ($P < 0.001$). The Met disappearance rates at 2, 4, 8, 12 and 24 h of incubation were 97.66 ± 0.066, 98.28 ± 0.140, 98.49 ± 0.096, 99.16 ± 0.072 and 99.95 ± 0.002 %, and the MCP concentrations were 0.13 ± 0.012, 0.15 ± 0.007, 0.16 ± 0.013, 0.22 ± 0.027 and 0.22 ± 0.007 mg/mL, respectively, both linearly increased with incubation time ($P < 0.001$). The NH_3-N concentrations of incubation liquids at 2, 4, 8, 12 and 24 h of incubation were 49.83 ± 0.655, 51.40 ± 1.266, 50.42 ± 0.846, 47.71 ± 3.726 and 54.18 ± 4.561 mg/100 mL, respectively, and no differences were found in the NH_3-N concentrations of incubation liquids among different incubation time points ($P > 0.10$). Taurine was undetectable at all time points of incubation.

Conclusions: The results indicated that the Met disappearance was high and fast. The unchanged NH_3-N concentration and the increased MCP concentration with incubation time suggested that Met was highly utilized for MCP synthesis rather than degraded. The results also indicated that rumen microorganisms appeared to be unable to use Met for taurine synthesis in *in vitro* rumen fermentation.

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11.

Does clustering of degradation data help to identify necessary modifications of the *Streptomyces griseus* protease test?

Hilft das Clustern von Abbaudaten bei der Identifikation notwendiger Modifizierungen des Streptomyces griseus Protease-Tests?

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Estimation of ruminal crude protein (CP) degradation is an essential part of feed protein evaluation. The Streptomyces griseus protease test provides a suitable *in vitro* method but feed-specific matrices might influence the degradation of feed protein byprotease. The objective of the study was to assess the suitability of the protease test for estimating effective degradation (ED) of CP compared to *in situ* results. Clustering was tried in an attempt, to identify clusters with typical nutrient characteristics. These cluster should be tested with specific additional enzymes to improve the vulnerability of the feed protein in the protease test.

Methods: The following 40 feedstuffs, for which *in situ* degradation data of feed CP studied for 48 and 72 h of incubation are available, were used: barley, wheat, corn, wheat bran, dried distillers' grains with solubles (DDGS), corn gluten feed (CGF), soybean meal (SBM), sunflower meal (SFM), 2 untreated rapeseed meals (RSM) (I-II), 2 expanded (et-) RSM (I-II), overtoasted (ot-) RSM, formaldehyde-treated (ft-) RSM, faba beans, 2 lupin grain varieties (I-II) native (n-) and toasted (t-), 3 pea grain varieties (I-III), sugar beet pulp (SBP) and 8 differently wilted grass silages (GS) ensiled with or without bacterial inoculant. The protease test was conducted according to [1] and protease solution (0.58 U/mL) was added at a ratio of 24 U/g feed true protein (TP). TP was determined according to the CNCPS as CP minus non-protein nitrogen (fraction A). Incubation times were adapted to reflect the *in situ* trial and were: 0, 2, 4, 6, 8, 16 and 24 h. After incubation, the solutions were filtered, residues dried and analysed for Kjeldahl nitrogen. The *in situ* degradation data were corrected for the amount of microbial nitrogen present in the feed residues according to [2]. Protein degradation parameters were estimated [3] and used to determine effective protein degradation (ED) for assumed ruminal passage rates of 0.02/h (ED_2), 0.05/h (ED_5) and 0.08/h (ED_8). Data were analyzed by t-test or Wilcoxon test. To compare *in situ* and *in vitro* ED, differences between ED_2, ED_5 and ED_8 estimated by both were expressed as degradation quotient (degQ): $degQ = ((ED_{in\ vitro} - ED_{in\ situ})/ED_{in\ situ})$. Clustering was done by single linkage method including degQ for the passage rates 0.02/h, 0.05/h and 0.08/h (SAS 9.4).

Results: Reliable estimation of ED *in vitro* was not possible in case of faba beans and corn due to implausible estimates of protein degradation parameters.Statistical analysis revealed no significant differences between *in situ* and *in vitro* ED_2 for et-RSM II, ft-RSM, pea grain variety III, and ED_5 for et-RSM II, ft-RSM, n- and t-lupin grain variety II, pea variety II, pea variety III and ED_8 for SFM, ft-RSM, t-lupin variety I, pea variety I and II. In general, the differences between *in situ* and *in vitro* ED were lowest in legume grains and highest in by-products and barley. The feedstuffs were clustered with respect to $degQ_2$, $degQ_5$ and $degQ_8$ as follows: cluster 1, soybean and et-RSM II; cluster 2, 2 untreated RSM; cluster 3, et-RSM I and all GS; cluster 4, pea grain varieties II and III; cluster 5, pea grain variety I and the lupin grain variety I; cluster 6, native lupin grain varieties I and II; cluster 7, wheat bran and DDGS; cluster 8, SBP and CGF. Some feedstuffs were arranged outside of any cluster: SBM, ft-RSM, wheat, ot-RSM, t-lupin grain variety II, SFM and barley.

Conclusions: Probably feed-specificmatrix and treatment effects resulted in almost diffuse clustering of feedstuffs regardless of their origin. The addition of carbohydrate-degrading enzymes seems to be required to reduce matrix effects and support protein degradation. However, clustering does not indicate any clear recommendation regarding a feed-specific different use of these enzymes.

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12.

The physical and chemical quality of peanut hull-based fermented total mixed ration using cassava leaves as the forage source

Physikalische und chemische Qualität einer fermentierten Gesamtmischration auf Erdnussschalenbasis unter Verwendung von Maniokblättern als Futtermittel

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During dry season, feed resources became a sensitive issue especially for ruminants. This allowed other potential feed resources to be used as an alternative, such as peanut hull and cassava leaves. Peanut hull can be used as fiber source because it has high crude fiber (CF) content, approximately 60% [1], while cassava leaves can be used as forage-based protein source due to its crude protein (CP) content (177-381 g/kg dry matter (DM)) [2]. To improve the nutrient quality, different feeding technologies could be combined such as total mixed ration (TMR) and fermentation. Total mixed ration (TMR) is one of the feedings technologies in which forages or roughages are well balanced with concentrates in proper proportion to meet the nutritional requirements of a certain animal stage without the addition of any feed additives other than water [3]. The aim of the study was to assess the physical and chemical qualities of a peanut-hull based fermented total mixed ration (PH-FTMR) which utilizing cassava leaves as forage source in various proportions.

Methods: Dried cassava leaves and peanut hull were finely grounded and combined with other ingredients such as copra meal, palm kernel meal, corn gluten meal, pollard, molasses, minerals, and urea to make the PH-FTMR. Formulated ration were divided into three groups of cassava leaves inclusion: 0% (T_1); 10% (T_2) and 15% (T_3) from total ration formulation (n=6). Feeds were fermented anaerobically for 14 days with the combination of mixed-culture bacteria (EM-4®) and fungi (Trichoderma viridae) as inoculum. Parameters observed were physical parameters (odor, texture, fungi appearances, and pH) and their chemical parameters: dry matter (DM), organic matter (OM), crude protein's (CP), crude fiber's (CF), and nitrogen-free extract (NFE) content. Statistical analysis was performed using SPSS software (IBM SPSS 25.0, Armonk, NY, USA). Data were analyzed using one-way ANOVA design. The significance of different for odor, texture, and fungi appearances were tested using Kruskall-Wallis whereas Duncan's Multiple Range Test (DMRT) was used to test pH and chemical qualities data.

Results: The results showed that the utilization of cassava leaves up to 15% rendered the odor to be less acid compared to T_1 and T_2 groups ($P<0.05$), although, the odor were between acid to slightly acid in all groups. The texture of the T3 group was slightly rougher compared to T_1 and T_2 groups ($P<0.05$). The results showed that the inclusion of cassava leaves up to 15% greater fungi appearances compared to T_1 and T_2 groups ($P<0.05$). The fungi itself was detected as Trichoderma viridae, the inoculum used for the fermentation process. The pH was not affected by inclusion of cassava leaves in all three groups in which range between 4.92 to 5.02. Furthermore, the inclusion of cassava leaves did not affect the DM and NFE content. However, the treatment increased the CP content consecutively 22.25% (T_2); 36.83% (T_3) compared to T_1 group ($P<0.05$) and also reduced the CF content consecutively 8.23% (T_2); 21.67% (T_3) compared to T_1 group ($P<0.05$).

Conclusions: The utilization of cassava leaves up to 15% provided the medium-good physical quality and improved the chemical quality, especially the greater CP content and the lower CF content compared to lower inclusion of cassava leaves.

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13.

Turnover of benzoxazinoids during aerobic exposure of maize silage

Turnover von Benzoxazinoiden während aerober Exposition von Maissilage

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Benzoxazinoids (BX) are specialized metabolites in Poaceae involved in plant nutrition, defense and environmental interactions. However, the role of phytocehemicals like BX in silage quality is widely unknown. After opening, silage is exposed to oxygen. Aerobic exposure triggers the development of deteriorating microbes that could be related to BX. This study investigated BX profiles in silage during aerobic exposure and the possible impact of BX presence on silage quality during aerobic exposure.

Methods: Maize genotypes of the wild type W22 line and a near-isogenic line of a BX deficient bx1 mutant bx1::W22 (referred to as bx1 [1]) were harvested 154 d after seeding and ensiled. At opening after 26 d, silage of the two maize varieties was loosely filled into 2-L polyethylene plastic containers covered with two layers of laboratory towel. Single-use NFC temperature data loggers (ETAG-1, Elitech Technology Inc.) were used to automatically record silage and ambient temperatures at 15 min intervals throughout the aerobic exposure for 14 d. Samples of silages (3 replicates per variety and sampling point) were obtained at d 0, 1, 2, 3, 5, 7, 10, and 14 of aerobic exposure. DM content and loss, silage pH, aerobic stability characteristics, and nutrient composition were measured. Fermentation products (lactic acid, acetic acid, propionic acid, n-butyric acid, isovaleric acid) and alcohols (1,2 propanediol, ethanol, n-propanol) were analyzed by HPLC. Yeasts and moulds were determined on YGC agar [2]. BX concentrations were determined [3] at d 0, 1, 3, and 5 of aerobic exposure. Temperature data from data loggers were averaged at 8 h intervals. Aerobic stability was assumed until silage temperatures exceeded the ambient temperature by more than 2K. For the statistical analysis (SAS, v9.4), mixed models were conducted using maize variety, time of aerobic exposure, and the variety × time interaction as fixed effects, and replicates within each genotype and sampling point as random effects. Significant effects were detected using Bonferroni-corrected t-tests ($P<0.05$).

Results: Aerobic stability was approximately 6 h less ($P=0.003$), and the interval to peak temperature around 5 h shorter in W22 compared to bx1 maize ($P=0.030$). Silage pH increased to a greater extent in W22 compared to bx1 silage ($P=0.026$). A greater proliferation of yeasts and moulds was detected in W22 compared to bx1 silage at d 5 and 7 of aerobic exposure ($P<0.05$). Maize genotype was not associated with the changes of fermentation products during the aerobic exposure ($P>0.05$). BX concentrations in bx1 maize silage were at a very low level compared to respective concentrations in W22 maize. Concentrations of DIMBOA (2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one) and HMBOA (2-hydroxy-7-methoxy-1,4-benzoxazin-3-one) in W22 maize silage started to decline at d 3 and further declined to nadir values close to the detection limit at d 5. In contrast, concentrations of MBOA (6-methoxy-1,3-benzoxazol-2-one) and BOA (1,3-benzoxazol-2-one) were increased at d 5 compared to d 1-3 in W22 ($P<0.05$).

Conclusions: While DIMBOA and HMBOA concentrations decreased with prolonged aerobic deterioration, concentrations of MBOA and BOA increased in silage produced from a BX-containing maize line. In particular, BX decrease silage aerobic stability, which was supported by a greater pH, and counts of yeasts and moulds in W22 compared to bx1 silage. The role of BX for silage quality and animal feeding warrants further investigations.

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14.

Comparison of gas production in the Hohenheim gas test obtained with wether faeces and rumen fluid as inoculum

Vergleich der Gasbildung im Hohenheimer Futterwerttest mit Einsatz von Hammelkot und Pansensaft als Inokulum

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The Hohenheim gas test (HGT) is a robust, cost- and time-saving assay to simulate rumen fermentation and determine metabolisable energy concentration and organic matter digestibility of feeds for ruminants. The official method [1] involves rumen fluid, commonly obtained from animals with a cannula in the rumen. Previous studies have suggested that using faeces from sheep may be an alternative to rumen fluid following adaptation of the assay, including an incubation period of 48 h with faeces instead of 24 h with rumen fluid [2]. The present study examined a wide range of common feeds for ruminants with the aim to determine the relationship between gas production obtained with faeces after 48 h and rumen fluid after 24 h.

Methods: A total of 90 feed samples for ruminants were used including cereal grains (n = 10), energy-rich co-products (n = 5), oilseed meals (n = 6), other protein-rich feeds (n = 12), legume grains (n = 6), roughages (n = 27), commercial compound feeds for dairy cows (n = 18), and total mixed rations (TMR) (n = 6). Each sample was incubated using rumen fluid from lactating cows for 24 h according to the standard procedure [1] and wether faeces for 48 h [2] with a minimum of 5 replicated syringes per feed and inoculum. A group of 3 wethers was fed with a standardized TMR in dry form for ad libitum intake. This TMR consisted of grass hay, straw, maize, rapeseed meal, and molasses and contained 70 % roughage. Immediately before starting the incubations, faeces were obtained from the rectum of the 3 wethers, pooled, and homogenized in a buffer medium. The ratio of faecal dry matter to buffer medium was 1:46 to 1:52. The relationship between gas production determined with faeces and rumen fluid was determined by linear regression analysis using the procedure REG of SAS 9.4 and the root mean square error (RMSE) was calculated. In both assays, gas production was expressed in mL / 200 mg dry matter (DM) of feed.

Results: The mean gas production was 52 mL/200 mg DM (min. – max. 21 – 83) after incubation using faeces and 56 mL/200 mg DM (18 – 85) after using rumen fluid. A strong linear relationship existed for the gas production determined either way. Including all feed samples, the linear regression equation for rumen fluid gas production (Y) and faeces gas production (X) was $Y = 1.12\ X - 2.31$ ($R^2 = 0.97$; RMSE = 5.5 %). A separate analysis for roughages (n = 27) and concentrates (n = 57) resulted in estimated linear regression equations of $Y = 1.20\ X - 7.76$ ($R^2 = 0.96$; RMSE = 5.5 %) for roughages and $Y = 1.02\ X + 4.39$ ($R^2 = 0.97$; RMSE = 4.1 %) for concentrates.

Conclusions: The results of this preliminary study confirmed a close relationship between the gas production obtained with faecal inoculum after 48 h and rumen fluid after 24 h when incubating different single and mixed feeds. This indicates a modified HGT using faeces might be developed to avoid the dependence on rumen-cannulated animals. The RMSE was lower in estimates for concentrates than roughages or the complete data set. This indicated that a differentiation between groups of feedstuffs needs more investigation in the further development of a modified assay.

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15.

In situ ruminal degradation of phytate and crude protein from soybean meal in lactating cows

In situ ruminaler Phytat- und Rohproteinabbau von Sojaextraktionsschrot bei Milchkühen

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In seeds of oil plants, phytate ($InsP_6$) is located in a protein-rich structure and a close relationship between ruminal $InsP_6$ and crude protein (CP) disappearance has been reported for rapeseed meal (RSM) and soybean meal (SBM) [1]. Production processes differ among industrial oil plants which influences ruminal CP degradation. For RSM, $InsP_6$ degradation was found to be affected by the processing condition similar to CP degradation [2]. The aim of this study was to investigate the variation of *in situ* ruminal $InsP_6$ degradation of SBM and its relation to CP degradation.

Methods: Nine commercial solvent-extracted SBM from Europe and South America were incubated in three rumen-fistulated lactating Jersey cows for 2, 4, 6, 8, 16, 24, 48, and 72 h based on a standard *in situ* procedure. The values of 0 h incubation were obtained by washing the feed samples without ruminal incubation. Bag residues were analysed for concentrations of inositol phosphates ($InsP_{3–6}$) and CP. Ruminal effective degradation of $InsP_6$ ($InsP_6ED$) and CP (CPED) was calculated for ruminal passage rates of 5 and 8%/h. Chemical protein fractions were determined according to Cornell Net Carbohydrate and Protein System. Data were statistically analysed in a one-factorial approach using SAS 9.4 with SBM variant as fixed effect and animal as random effect. Statistical significance was declared at $p < 0.05$.

Results: $InsP_6$ concentration of SBM ranged from 12.1 to 16.3 g/kg DM and $InsP_5$ concentration from 1.9 to 3.6 g/kg DM. Traces of $InsP_4$ was detected (0.1–0.4 g) but $InsP_3$ was not detected in any SBM. Overall, concentrations of $InsP_6$ and $InsP_5$ in the bag residues decreased between 2 and 24 h of incubation, with $InsP_6$ comprising the most abundant inositol phosphate at any incubation time. However, the SBM samples varied considerably in the reduction rates of $InsP_6$ and $InsP_5$, wherein the greatest variation was observed at 24 h of incubation ($InsP_6$: 0.7–9.0 g/kg DM; $InsP_5$: not detectable–1.5 g/kg DM). After 48 h of ruminal incubation, $InsP_6$ concentration was reduced to less than 1 g/kg DM and $InsP_5$ was not detectable in the majority of bag residues. $InsP_4$ was detected at very low concentration (0.1 g/kg DM) until 2 h of ruminal incubation but not detected in the majority of the samples after 4 h of incubation. $InsP_3$ was not detectable in any bag residue. Calculated ruminal degradation of $InsP_6$ and CP differed significantly among SBM ($p < 0.05$), with 65–86% and 45–72% for $InsP_6ED_5$ and $CPED_5$, respectively ($InsP_6ED_8$: 55–81%; $CPED_8$: 32–61%). Independent of assumed rumen passage rates, significant correlations were found between $InsP_6ED$ and CPED (≥ 0.881) and between $InsP_6ED$ and chemical protein fractions A, B1, B3, and C (A: ≥ -0.761; B1: ≥ 0.797; B3: ≥ -0.846; C: ≥ -0.932) of SBM ($p < 0.05$). Linear regression equation for $InsP_6ED$ (y) depending on CPED (x) was $y = 0.74x + 34.1$ ($R^2 = 0.79$; RMSE = 3.76) for 5%/h and $y = 0.85x + 29.4$ ($R^2 = 0.78$; RMSE = 4.73) for 8%/h rumen passage rate, respectively. Equations to estimate $InsP_6ED$ from chemical protein fractions A, B1, B3, and C (all in % of CP) were calculated as $InsP_6ED_5$ (%) = 96.66 − 0.58*A + 0.30*B1 – 0.26*B3 – 12.87*C ($R^2 = 0.95$; RMSE = 1.74) and $InsP_6ED_8 = 91.32 − 0.79$*A + 0.48*B1 – 0.09*B3 – 16.93*C (R2 = 0.96; RMSE = 2.07).

Conclusions: Ruminal $InsP_6$ degradation varies markedly among SBM from different origin and is closely related to CP degradation. Results suggest that $InsP_6ED$ values may be predicted from CPED values and from chemical protein fractions. This warrants confirmation in a larger data set and independent validation.

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16.

In situ ruminal crude protein degradation and *in vitro* intestinal digestibility of UDP from soybean meal

In situ Rohproteinabbau im Pansen und in vitro Verdaulichkeit des UDP von Sojaextraktionsschrot

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Common milk production systems rely on high nutrient dense diets including a large proportion of protein feeds to meet the requirements of dairy cows. Soybean meal (SBM) is such a protein feed with high concentration of rumen undegradable protein (UDP). Replacement of SBM by alternatives is one goal in ruminant nutrition research; however, because transformation is slow and SBM widely used globally, it is necessary to have reliable protein values for currently available SBM. The aims of the present study were to determine the variation in ruminal degradability of CP and the intestinal digestibility of rumen undegradable protein (IDUDP) of contemporary SBM.

Methods: The study was performed with 17 SBM sampled from different countries with a wide range in their chemical composition. Rumen degradation was determined *in situ* according to [1] with minor modifications. The SBM were weighed into bags (50 µm pore size). Three lactating ruminallly cannulated Jersey cows were used for rumen *in situ* incubations (0, 2, 4, 6, 8, 12, 16, 24, 48, and 72 h). A minimum of 9 measurements were carried out for each point in time. The CP concentration of the SBM and bag residues were analyzed by NIRS using a calibration developed for this application. Degradation parameters a (soluble), b (degradable), c (degradation rate) and effective degradation ($EDCP_6$) for a rumen outflow of k=0.06 h-1 were calculated according to [2] including a lag-phase. The UDP was calculated as UDP6 (%) = 100 – $EDCP_6$ (%). *In situ* residues after 16 h were used to determine IDUDP according to the three-step enzymatic method [3]. Protein fractionation was based on the Cornell Net Carbohydrate and Protein System (CNCPS) and protein fractions A (non-protein nitrogen), B1 (fast degradation rate), B2 (intermediate degradation rate), B3 (slow degradation rate), and C (unavailable) were calculated.

Results: Degradation of CP started after a mean lag phase of 1.6 h (Min-Max: 1.1-2.0 h) and was almost complete after 72 h. *In situ* a and b fractions varied from 1 to 11% and 89 to 99%, respectively. Degradation rates averaged 10.1%/h and varied from 4.7 to 13.9%/h. The $EDCP_6$ ranged from 40 to 68%. Although considerable variation in UDP6 (32-60%), the IDUDP was on average high (93%) with relatively small variation (87-96%). Most of the protein from SBM was assigned to protein fraction B2 (70.1-82.0%) whereas fraction B3 showed high variation between samples (2.9-20%) and C was small overall (0.8-1.2%). Correlations between the ruminal degradation rate and $EDCP_6$ with the protein fraction B1 were positive ($P < 0.001$) and negative correlations ($P < 0.05$) were detected with B3 and fraction C.

Conclusions: *In situ* CP degradation and UDP concentration vary widely between SBM from different origin. However, IDUDP was high for all samples independent of the UDP content and origin. This indicates that high UDP concentration of SBM can be achieved without negative effects on intestinal protein digestibility. Significant correlation of UDP with chemical protein fractions suggest that they can be used to predict ruminal degradation of CP and UDP of SBM for practical application.

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17.

Lingual and central taste perception of dairy cows and its modulation by N-arachidonoylethanolamide

Die linguale und zentrale Geschmackswahrnehmung von Kühen und deren Modulation durch N-Arachidonylethanolamid

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In the process of taste perception, signals from tongue taste receptors are transmitted to the gustatory cortex and the reward system, the latter including the amygdala and nucleus accumbens (NAc). Thus, the reward system motivates or prevent the animal to ingest feed with a certain taste. Feed rewards and feed intake are modulated by the endocannabinoid and opioid systems of the limbic system. In our previous studies, we found that the plasma concentration of the endocannabinoid N-arachidonoylethanolamide (AEA) increases from late gestation to early lactation, that early lactating cows prefer sweet tasting feed, and that AEA administration enhances intake of sweet tasting feed [1, 2]. Here, we hypothesize that AEA administration increases the expression of taste-related genes in the tongue and the expression of genes encoding endocannabinoid and opioid receptors in the amygdala and NAc of early lactating dairy cows.

Methods: Eight half-sibling Holstein dairy cow pairs were comparable in the lactation number, age, and expected calving date. After calving, half-siblings of each pair were randomly assigned to either AEA (n = 8) or control (CON) group (n = 8). The AEA group received intraperitoneal injections of 3 µg/kg body weight AEA (in 50 ml 0.9% NaCl); the CON group received 50 ml 0.9% NaCl, each day at 07.00 a.m. from Mondays to Fridays. On day 30 postpartum, cows were sacrificed to obtain tissue from tongue epithelium, as well as to take the amygdala and NAc of the left and right hemisphere. Tongue tissue was snap frozen in liquid nitrogen and brain tissue stored in RNAlater, both at -70°C until analysis. RNA was extracted, reverse transcribed, and subjected to real-time quantitative PCR. Data were analysed using the linear mixed model (LMM, lmer function, lme4 package) in R (4.2.0). Models contained group (AEA, CON) as fixed factor and sire of half-siblings as a random factor. The model for the brain data included additionally hemisphere (left, right), and the interaction between group and hemisphere as fixed factors.

Results: The mRNA expressions of the endocannabinoid receptor 1 CNR1 and G protein-coupled receptor 55 (GPR55), as well as the taste receptor detecting sweet taste (type 1 member 2; TAS1R2) and the G protein subunit alpha transducin 3 (GNAT3) in tongue epithelium were not different between groups. The mRNA abundances of CNR1 and the opioid receptor delta 1 (OPRD1) were lower ($P < 0.05$), whereas the opioid receptor mu 1 (OPRM1), kappa 1 (OPRK1), and nociceptin receptor 1 (OPRL1) expressions in the left amygdala were not affected in AEA relative to CON cows. In the right amygdala, OPRK1 mRNA expression was lower in AEA than CON cows ($P < 0.05$), but the CNR1, OPRL1, OPRM1, and OPRD1 transcript abundances were comparable between groups. There were no effects of AEA treatment on endocannabinoid and the opioid receptor mRNA abundances in the right and left NAc.

Conclusions: The reduced CNR1 mRNA expression in the left amygdala after repeated AEA administrations is associated with changes in taste perception leading to a higher preference for sweet tasting feed. Furthermore, the reduced mRNA expressions of kappa and delta opioid receptors in the amygdala after AEA administration indicates the involvement of endocannabinoid-opioid cross-modulation in taste preference of cows.

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18.

The nitrogen emission mitigation potential of willow leaves (*Salix spp.*) supplemented to cattle on pasture

Das Stickstoffemissionsminderungspotenzial von Weidenblättern (Salix spp.) als Ergänzungsfuttermittel in der Weiderinderhaltung

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Agroforestry systems offer numerous economic and ecological advantages compared to conventional agricultural systems involving the protection of climate, water, soil and biodiversity. The nutrient pattern of tree leaves as co-product of agroforestry systems is considered to have a high potential in ruminant nutrition [1]. Moreover, tree leaves contain tannins which, at a certain concentration, are described to reduce nitrogen (N) emissions without influencing animal performance [1]. However, previous studies investigating the emission-reducing effects of tannins focused primarily on leaves from tropical tree types and less from tree types found in temperate zones. Therefore, the objective of the present study was to analyze the nutritional and N emission mitigation potential of willow (*Salix spp.*) tree leave supplementation to cattle kept on an experimental pastoral system.

Methods: Eight German Holstein bull calves were weaned at 12 week of age. Calves were adapted to the barn and had free access to pasture for one week. During the subsequent experimental period, calves with access to pasture or fresh gras cuttings were fed two supplements containing concentrate and either dried willow leaves (SALIX) or alfalfa hay (CON) in a crossover design. Supplements were formulated isocaloric (9.7 MJ metabolizable energy/kg dry matter (DM)) and isonitrogenous (134 g crude protein/kg DM). Calves were weighed every three days to adjust the daily amounts of SALIX and CON intake to 2.2% of body weight. The experimental period included a two-week feed adaptation and a four-day collection period. For urine collection, calves were kept in tie-stall and equipped with a urinal. Furthermore, rumen fluid was sampled by an oral probe. Intake of gras cuttings and supplements (SALIX or CON) as well as urine excretions were quantified and subsampled for four days. Subsequently, calves were transferred to the opposite supplement. Urinary N metabolites were analysed by high performance liquid chromatography. Urinary N concentration was analysed according to the Dumas method. The rumen fluid ammonia concentration was analyzed according to the Conway method. Statistical analysis was carried out using the SAS software for Windows, version 9.4 (Copyright, SAS Institute Inc., Cary, NC, USA). Data was analysed by a MIXED MODEL procedure including the repeated statement diet and the fixed effects diet (SALIX or CON) and block. Results were considered statistical significant at $P < 0.05$ and tendencies between $0.05 < P < 0.10$.

Results: Neither DM intake, body weight, average daily gain (1.24 ±0.06 kg/d), nor urinary N or uric acid excretions differed between experimental groups. The SALIX group revealed higher urinary hippuric acid concentrations ($P < 0.01$) and excreted more than twice the amount of hippuric acid ($P < 0.01$) compared to calves fed the CON diet. In addition, the SALIX group tended to have higher urinary concentrations of allantoin and purine derivatives but lower urinary urea concentrations and excretions than the CON group ($P < 0.1$). Feeding the SALIX diet reduced the ammonia concentration in rumen fluid ($P < 0.01$).

Conclusions: Willow leave compared to hay supplementation to calves on pasture does not have any negative effects on animal performance, suggesting that willow leaves may be a suitable forage in cattle nutrition. Diminished rumen fluid ammonia concentrations and higher urinary purine excretion indicate higher microbial N flow to the small intestine and rumen microbial N utilization efficiency in the SALIX group [2]. Greater urinary hippuric acid excretions may be due to higher intake of benzoic acid derivatives contained in willow leaves. Hippuric acid may inhibit nitrous oxide emissions from soil [3], but if the urine from the SALIX group reduces nitrous oxide and/or ammonia emissions remains to be investigated in future studies.

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19.

Effect of the age of broilers on the precaecal digestibility of amino acids of pea grains

Effekt des Alters von Broilern auf die praecaecale Verdaulichkeit von Aminosäuren aus Erbsenkörnern

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The effect of the age of broilers on the precaecal digestibility (pcD) of amino acids (AA) of different feed ingredients is not sufficiently clarified [1]. Previous own studies showed an enhancement in pcD of AA for soybean meal between 2 and 4 weeks of age. [2]. In contrast in wheat the pcD of AA was higher in 2 than 4 weeks old broilers [1]. The aim of the present study was to clarify the influence of age on the pcD of AA of pea grains but for older broilers commonly used in organic farming at 5, 8 and 11 weeks of age and both sexes.

Methods: Pea grains (crude protein, 208; crude fat, 12; crude fibre, 54 g/kg; as fed basis) of organic origin were tested. The grains were included at levels of 0, 100 and 200 g/kg into a basal diet which contains maize, wheat gluten, soybean meal and maize starch as main ingredients at the expense of starch. TiO_2 was included as indigestible marker (5 g/kg). Females and males (Isa 757) were reared in two separate trials with a commercial starter feed during the first weeks. The experimental diets were offered ad libitum for 5 days. Ten pens per sex with 4 to 7 birds were allocated to each treatment. Finally at week 5, 8 and 11 all birds were sacrificed by asphyxiation with CO_2, the content of the medial and terminal ileum was flushed out with distilled water, pooled for all birds of a pen and immediately frozen. Total N, AA and TiO_2 were analysed in diets and freeze-dried ileal digesta. For measuring the pcD of AA, the feed intake of the last 24 h prior to digesta removal was recorded. The pcD of AA of the test ingredient was determined by linear regression analysis [3]. Differences in pcD of N and AA within males and females were tested by using the procedures REG and MIXED (SAS 9.4, SAS Institute Inc., Cary, NC, USA). The level of significance was at $P < 0.05$.

Results: The mean feed intake for males varied between 93 (10 % peas, week 5) and 226 g/d (20 % peas, week 11; $P < 0.001$) and 65 (10 % peas, week 5) and 164 g/d (10 % peas, week 11; $P < 0.001$) for females. The mean pcD of essential AA (arginine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, valine) ranged between 94 (week 5) and 91 % (week 11) for males. For females a variation between 90 (week 5) and 96 % (week 11) was measured. The essential AA with the highest pcD was methionine (99/100 %, males/females, week 11). Contrary, tryptophan showed the lowest pcD with 83 % for both sexes (males, week 11; females, week 5). With the exception of phenylalanine for males (week 5, 94 % and week 11, 88 %; $P = 0.038$) and methionine for females (week 5, 94 % and week 11, 100 %; $P = 0.026$) the pcD of the essential AA was not significantly influenced by the age ($P > 0.05$).

Conclusions: The pcD of essential AA of pea grains was barely influenced by the age of the broilers. Both males and females digested AA at a high level. It seems that in broilers the capacity of pcD of AA is most widely developed at least with an age of 5 weeks.

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20.

Evaluation of three different rapeseed varieties for broiler nutrition

Bewertung von drei verschiedenen Rapssorten für die Ernährung von Broilern

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This study evaluated the impacts of different inclusion levels of rapeseed cakes (RSC) with various fiber content (black, brown and yellow varieties) in broiler diets on growth performance and nutrient digestibility.

Methods: Chemical composition of the rapeseed cakes was determined. Six starter (d 1-21) and six grower (d 22-35) diets were produced by 10 and 20% inclusion of three different rapeseed cakes (black, brown, and yellow) as partial replacements for soybean meal. The experimental diets were formulated to be isocaloric and isonitrogenous. The grower diets contained 3 g titanium dioxide per kg feed (Sigma Aldrich, St. Louis, MO) as an indigestible marker to allow for determination of the apparent ileal digestibility coefficient (CAID) of nutrients. In total, 480 one-day-old broiler chicks were randomly distributed to 48 pens and the experimental diets were randomly assigned to birds within pens (10 birds per pen and 8 pens per diet). The trial lasted 35 d. Growth performance variables were recorded weekly and at the end of the experiment, AID of nutrients was measured. Data were subjected to ANOVA using the GLM procedure.

Results: The black RSC had the highest fat (19.62%) and fiber (7.55%) content, and the yellow RSC had the lowest content of fat (13.66%) and fiber (6.11%). However, crude protein (39.04%) content of the yellow RSC was higher than the brown one (35.99%), and the brown RSC had higher protein content than the black RSC (31.51%). During the grower period and entire experiment, body weight gain (BWG) and feed conversion ratio (FCR) of birds fed the brown (1,118 g and 1.582 respectively) and yellow (1,123 g and 1.608 respectively) RSC were better than birds receiving the black (1,068 g and 1.658 respectively) RSC ($P \leq 0.05$). The type of RSC had no effect on BWG and FCR of birds during the starter period. Moreover, feed intake of broilers was not affected by the type of RSC during the trial. Inclusion levels of RSC did not affect performance variables in the study ($P > 0.05$), while increasing the inclusion level of RSC in broiler diets reduced CAID of all the nutrient except for fat, Ca, Glu, Gly, Val, His and Cys ($P \leq 0.05$). CAID of fat in diets with the brown and yellow RSC was better than those with the black RSC (0.834 and 0.822 vs. 0.802 respectively), while CAID of P in diets with the brown and black (0.499 and 0.499 respectively) RSC was better than those with the yellow (0.443) RSC ($P \leq 0.05$). CAID of Glu (0.915 vs. 0.906, 0.906 respectively), Val (0.846 vs. 0.833, 0.827 respectively), Leu (0.860 vs. 0.846, 0.841 respectively), Ile (0.880 vs. 0.869, 0.865 respectively), Lys (0.894 vs. 0.879, 0.874 respectively) and total amino acids (0.871 vs. 0.859, 0.856 respectively) was higher in the diets containing the brown RSC compared with diets containing the black and yellow RSC ($P \leq 0.05$). Diets with the brown RSC showed higher CAID of Tyr (0.857 vs. 0.839) and Phe (0.883 vs. 0.868) compared with those containing the yellow RSC ($P \leq 0.05$), while CAID of these two AA in the black RSC diets (0.845 and 0.873 respectively) was similar to diets with the yellow and brown RSC. CAID of Met was lower in diets with the yellow RSC (0.924) compared with diets containing the black (0.932) and brown (0.935) RSC ($P \leq 0.05$).

Conclusions: Increasing inclusion level of rapeseed cakes from 10 to 20 percent in the experimental diets had no negative impact on growth performance of broilers, while broilers receiving diets with low fiber rapeseed cakes (e.g. brown and yellow) performed better than those receiving diets with black rapeseed cake. However, CAID of nutrients in the experimental diets was not completely in line with what was observed for growth performance of broilers. Increasing the inclusion level of RSC in broiler diets impaired CAID of crude protein, P and most of the amino acids, while diets with the brown rapeseed cake, generally showed higher nutrients digestibility compared with diets containing the black and yellow rapeseed cake.

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21.

Neutral detergent fibre concentration in different genotypes affects *in vitro* gas production for oat and rye grain, but not for barley grain

Die Neutral-Detergenzien-Faser-Konzentration in unterschiedlichen Genotypen beeinflusst die in vitro Gasproduktion von Hafer und Roggen, aber nicht von Gerste

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Dietary fibre is defined as component (mainly nonstarch polysaccharides (NSP)) resistant to enzymatic digestion and available for microbial fermentation primarily in the hindgut of non-ruminants. The rate and extent (kinetic) of fermentation of dietary fibre is important because the fermentation mainly produces short-chain fatty acids relating to animal health. In pig diets, cereal grains are primarily used as energy source based on the starch concentration, however, because of their high inclusion rate cereal grains also contribute high amounts of NSP. We hypothesised that cereal grain genotypes varying in neutral detergent fibre (NDF) concentration would affect *in vitro* fermentation characteristics thereby gas production *in vitro*.

Methods: Five genotypes each of three cereal grain species were tested(barley: ACK 2927, Lomerit, Sandra, Semper, Malwinta; oat: Buggy, Energie (EU), Zorro (EU), Max, Moritz; rye: Palazzo, Dankowski Diament, Cantor, Hellvus, Boresto). The modified Hohenheim gas test was used with fresh pig faeces as inoculum to assess the gas production for 48 hours. Four barrows (initial body weight 37.3 ± 2.3 kg) were used as donor animals and were fed a standard commercial diet devoid of antibiotics. The pooled inoculum was diluted 1:57 in buffer solution. In 3 batches, faecal inoculum was incubated at 39°C in a fermentation glass syringe (100 ml volume) with 200 mg ground (1 mm) sample in duplicates. Gas production was recorded at 0, 2, 5, 8, 12, 18, 24, 36, and 48 hours. Model parameters such as potential and rate constant of gas production were estimated by an iterative least squares procedure (GraphPad Prism 5.0). MIXED models analysed the data separately for each cereal grain with genotype as fixed effect and batch as random effect. Predicted values were used to analyse linear and quadratic effects of NDF concentration in cereal grain genotypes on fermentation kinetic variables and gas production.

Results: On dry matter, NDF concentration in barley, oat and rye grain ranged between 15.2 to 20.9%, 26.1 to 34.1%, and 12.6 to 17.2%. Gas production was not affected ($P > 0.05$) for barley grain. For oat, gas production for timepoints 8, 12, 18, 24, 36, and 48 hours and the potential gas production was greater ($P < 0.001$) for Buggy compared to Zorro and Energie (EU), and for Max and Moritz compared to Zorro and Energie (EU). Increasing NDF concentration in oat grain quadratically decreased ($P < 0.001$) gas production for timepoint 8, 12, 18, 24, 36, 48 hours and potential gas production. For rye, gas production for timepoint 18, 24, 48 hours and potential gas production were linearly decreased ($P < 0.05$) with increasing NDF concentration in rye grain. The rate constant of gas production did not differ ($P > 0.05$) within genotypes for barley, oat, and rye grain.

Conclusions: For rye and oat grain, differences in fermentation kinetics variables and gas production among genotypes differing in NDF concentration could be attributed to their physical-chemical characteristics. Gas production was affected by NDF concentration for oat and rye, but not for barley which can be attributed to constituents of dietary fibre that are not detected by NDF analysis such as soluble NSP.

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22.

Reactive lysine: An *in vitro* parameter to predict the concentration of standardized ileal digestible lysine in DDGS samples based on trials using cecectomized roosters

Reaktives Lysin: Eine in vitro Methode zur Vorhersage des Gehalts an standardisiert praecaecal verdaulichem Lysin von DDGS basierend auf Fütterungsversuchen mit caecaectomierten Hähnen

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For optimal animal performance, information regarding the standardized digestibility of amino acid (SDD AA) and the content of metabolizable energy (TME_N) of feedstuffs are prerequisite for diet formulation, to cover the nutritional requirements and reduce negative environmental consequences. Furthermore, one major challenge in the future is to reduce the strong competition for food between humans and monogastric livestock. Therefore, the integration of regional by-products from the food, feed and fuel processing industry like dried distillers' grains with solubles (DDGS), can be a strategy in diet formulation for monogastric animals, contributing to a more sustainable livestock production. However, a too extensive heat treatment during DDGS processing may provide favourable conditions for protein denaturation, Maillard reaction and degradation of heat-labile amino acids, which may negatively affects animal performance [1]. In this regard, there is increasing focus of today's scientific work for identification of robust laboratory methods (*in vitro*) which may highly correlate with *in vivo* data to correspond with principles of the 3Rs (Replacement, Reduction and Refinement). Therefore, the objective of the present study was to develop a regression equation based on *in vitro* and *in vivo* measurements to predict the concentration of SDD lysine (Lys) and metabolisable energy (TME_N) for poultry nutrition. It was hypothesised, that strongest correlation between SDD Lys as well as TEM_N and reactive lysine (rLys/Lys) might exist.

Methods: Seven samples of DDGS from a dry-grind ethanol plant were tested in the experiment. DDGS samples mainly based on wheat, corn and triticale. The SDD of AA and TME_N in each source of DDGS was measured using the applied cecectomized rooster method [2]. The content of rLys was analysed according to [3]. The DDGS samples were analysed for crude protein (CP) and crude fibre (CF). The content of AA in DDGS and faeces samples were quantified using HPLC. The results of *in vivo* SDD Lys and TME_N in each source of DDGS were correlated with the content of Lys and rLys, rLys/Lys (relative amount of rLys per Lys, %) as well as the concentration of Lys per 100 g CP using PROC CORR of SAS.

Results: CP and CF of the seven DDGS samples ranged from 281 up to 329 (±17.8) g kg-1 and 85.8 up to 117 (± 11.1) g kg-1. The content of Lys ranged from 7.3 up to 10.8 (±1.3) g kg-1, while calculated concentration of Lys per 100 g CP was between 2.35 and 3.50 (±0.44) g kg-1. Based on cecectomized roosters´ feeding experiment, the SDD of Lys ranged from 48.9 up to 82.8 (±12.2) % and TME_N from 13.3 up to 14.8 (±0.58) MJ kg-1. Regarding TME_N, no correlation with CP ($r= -0.082$, $p= 0.861$), but with Lys ($r= 0.801$, $p= 0.03$) as well as the concentration of Lys per 100 g CP ($r = 0.781$, $p= 0.04$) was observed. The strongest correlation for TME_N was found between the content of rLys ($r = 0.814$, $p= 0.01$) and CF ($r= -0.883$, $p= 0.01$). The content of Lys correlated with *in vivo* SDD Lys ($r= 0.786$, $p= 0.04$), while Lys concentration per 100 g CP was even stronger correlated with *in vivo* SDD Lys ($r= 0.848$, $p= 0.02$). The strongest correlation was detected for *in vivo* SDD Lys and rLys/Lys, which indicates that greater amounts of Lys are digested by roosters when the ε-NH_2 group of Lys is not bound to reducing sugars ($r= 0.973$, $p< 0.001$).

Conclusions: The rLys/Lys seems to be an alternative parameter to evaluate DDGS quality in addition to AA digestibility and TMEN using cecectomized roosters. Further research should be done to back up the results of the present preliminary study.

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23.

Rye bran as a component in diets for lactating sows – effects on sow and piglet performance

Roggenkleie als Mischfutterkomponente für laktierende Sauen – Auswirkungen auf die Sauen- und Ferkelleistung

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Rye is attracting more and more attention as a feed component. Its higher drought resistance and adaptation to nutrient-poor soils make it an attractive cereal, especially regarding climate change and fertilizer restrictions. Breeding efforts in rye significantly reduced the risk of ergot contamination, allowing rye to be used again for breeding animals [1]. Rye bran, as a by-product of the food industry, can be characterized as a cost-effective and resource-saving feed component. It has a high content of dietary fiber, vitamins, minerals, and other bioactive substances [2]. Rye contains more non-starch polysaccharides (NSP) compared to other cereals. Rye bran, in particular, contains high levels of NSP, which promotes fermentation to short-chain fatty acids (SCFA), especially butyrate. The volatile fatty acid butyrate is said to have a positive effect on the intestinal health of pigs and also reduces the prevalence of Salmonella in the intestine [3]. Concerning the use of rye bran to reduce Salmonella prevalence in piglet production, the effects of rye bran as a feed component on sow and piglet performance have to be evaluated first.

Methods: Feeding trials were conducted on a conventional piglet production farm (1000 sows). A commercial feed containing wheat, barley, soybean meal and wheat bran as main components was used as control diet (C). On this basis, an isonitrogenous and isoenergetic (13.4 MJ ME/kg; 17.5 g/kg oS crude protein) experimental diet (R) with 15% rye bran was calculated, which no longer contained wheat bran. An amount of 15% rye bran corresponded to the maximum possible inclusion rate in order to maintain the required energy content in the feed. The feeds were analyzed for crude nutrients using the Weender analysis according to the LUFA standard. Mycotoxin analysis was performed by high performance chromatography (HPLC) whereby Ergotamine served as an indicator for the ergot alkaloids. The experiment and thus also the feeding of the sows began one week before the farrowing date and ended at weaning of the piglets. The body weight (kg) (n(C/R)=43/42) and backfat thickness (mm) (n(C/R)=19/19) of the sows were measured one week before farrowing and at weaning. The backfat thickness of the sows was determined by ultrasound sonography. The loss of weight and backfat thickness during lactation was calculated from the difference between the weight one week before farrowing and at weaning. The weight (kg) of individual piglets from a total of 40 litters (n(C/R)=20/20) was determined at birth (d0: n(C/R)=327/334), and on days 1(d1: n(C/R)=327/334), 7(d7: n(C/R)=288/289), 14(d14: n(C/R)=281/283) and 20(d20: n(C/R)=280/271) of life. Statistical evaluation was conducted with SAS Enterprise Guide® (t-test) and $p < 0.05$ was considered significant.

Results: The inclusion of rye bran into the compound feed had no effect on the feed intake of the sows. No significant differences regarding body weight loss (C 29.51±12.95kg, R 31.13±10.18kg) and the loss of backfat thickness (C 2.33±1.24mm, R 2.24±1.96mm) of the sows occurred during lactation. The body weight of the suckling piglets in the experimental group showed no significant difference at any timepoint compared to the control group (d0: C 1.26±0.35kg, R 1.26±0.29kg // d1: C 1.33±0.36kg, R 1.32±0.33kg // d7: C 2.21±0.55kg, R 2.21±0.52kg // d14: C 3.70±0.90kg, R 3.76±0.88kg // d20: C 4.94±1.21kg, R 5.08±1.25kg). The piglet gains calculated from this also showed no significant differences.

Conclusions: The results of the study showed that rye bran as a feed component had no significant effect on sow and piglet performance. Therefore, rye bran can be used as a cost-effective by-product with levels up to 15% in the diet without causing obvious disadvantages. More research is needed to demonstrate the potential positive effects on Salmonella prevalence in piglet production, and also on the pig microbiome.

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24.

Effects of chemical treatment of barley straw with sodium hydroxide or urea on chemical characteristics, *in vivo* digestibility and *in vitro* gas production in ruminants

Effekte einer chemischen Behandlung von Gerstenstroh mit Natronlauge oder Harnstoff auf die in vivo Verdaulichkeit und in vitro Gasproduktion für Wiederkäuer

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Forage shortage for different reasons is a well-known problem and previous work has extensively addressed the topic of straw treatment in this concern [1]. We hypothesized that the treatment of straw with NaOH or feed grade urea adapted as practicable procedure for modern farm conditions affect the *in vivo* digestibility and *in vitro* fermentability of a straw-containing total mixed rations (TMR) and straw as single feedstuff (*in vitro* only), respectively.

Methods: Barley straw was treated with caustic soda microbeads (60 g NaOH/kg dry matter [DM]), dissolved in water. For urea treatment, urea (90 %) was applied with 40 g/kg DM and mixed for 5 min. With both treatments, the straw was remoistened to 600 g DM/kg. The treated straw was stored 14 d at ambient temperature in January 2021 (0.8 °C on monthly average) (NaOH treatment) and 25 °C (urea treatment) and then frozen to – 20 °C until analysis. To characterize lignification, ^{13}C solid state NMR and FTIR analysis were performed using a Bruker Avance III HD 300 MHz spectrometer. Standardized digestibility trials with wethers were performed [2] using TMR containing 18 % (dry matter basis) of either native or treated straw. *In vitro* gas production with all single straw variants, and 0, 10 and 20 % (dry material) in a lab-scale TMR was measured using the ANKOM RF Gas Production System and *in vitro* organic matter digestibility was calculated (IVOMD) [3]. Statistics was performed using SAS (9.4).

Results: NaOH treatment increased crude ash and non-fibrous carbohydrates whereas neutral detergent fiber (aNDFom) and hemicellulose decreased. In urea treated straw, NH_3-N and crude protein increased, but acid detergent lignin decreased. ^{13}C NMR and FTIR analyses showed that the global structure and crystallinity of the cell wall carbohydrates and lignin content were not altered and depolymerization of lignin did not occur.NMR signals assigned to acetyl groups were altered ($P < 0.05$) indicating straw treatments disrupted linkages between hemicelluloses and lignin. Acetates signal was affected which can be assigned to linkages between ferulic acids and hemicelluloses (arabinoxylans). FTIR spectra after straw treatments were also altered suggesting removal of hemicelluloses or lignin related compounds and conjugated ketone (phenyl-carbonyl) or the removal of ferulic and p-coumaric acid acetyl groups. *In vivo* digestibility of aNDFom, acid detergent fiber, hemicellulose and cellulose increased in the TMR by approximately 10%-points following NaOH treatment ($P < 0.05$) and metabolizable energy likewise rose ($P < 0.05$). The inclusion of urea treated straw did not affect digestibility ($P < 0.05$). Straw as pure substrates *in vitro* showed elevated gas production and IVOMD (from 0.31 to 0.51; $P < 0.05$) for NaOH treatment but only tended to increase IVOMD following urea treatment (0.41; $P > 0.05$). As part of TMR, straw treatments had no distinct effect on *in vitro* gas production or IVOMD. Concentrations of CH_4 and CO_2 in the *in vitro* produced gasses were likewise not affected ($P > 0.05$).

Conclusions: *In vivo* and *in vitro* results confirm NaOH treatment being a reliable method to elevate digestibility and energy content of straw for ruminants [see also 1]. The effort is partly limited by the elevated crude ash content. Chemical characterization reveals treatments might have released fermentable cell wall components from lignin-associated bonds and as a result, straw fiber might potentially be better fermented in the rumen but release phenolic compounds which may lead to higher renal energy losses via hippuric acid.

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25.

Age-dependent gut microbiota of broilers and turkeys fed with different dietary levels of P, Ca, and phytase

Altersabhängige Darmmikrobiota von Broilern und Puten, die mit unterschiedlichen Mengen an P, Ca und Phytase gefüttert wurden

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The supplementation of mineral Ca and P in the form of monocalcium phosphate (MCP) is a common practice in feed formulation and helps to fill the lack of available P and Ca in the feed. P in plants is primarily stored in the form of phytate ($InsP_6$), and its digestibility by the animals can be improved by adding the enzyme phytase to the feed, which can increase the release of $InsP_6$-bound P. This work aimed to investigate whether different levels of P and Ca or phytase in the feed affect the microbial composition in the digestive tract of turkeys and broilers and to examine the effect of age under the same dietary and environmental conditions for both species.

Methods: A total of 480 Ross 308 broilers and 480 B.U.T. 6 turkeys were used in this study. Upon the arrival of the hatchlings at the experimental station, birds were raised under the same conditions (floor pens, deep litter bedding, and diet according to feeding recommendations). On days 14 and 35, half of the animals were moved in groups of ten to 48 perforated floor pens and one of four experimental diets was randomly allocated to each pen. Both species received the same experimental diets that involved two levels of phytase supplementation (0 (Phy-) and 1500 FTU/kg (Phy+)) and two levels of P and Ca (with and without MCP and limestone;CaP+ /CaP-) using a Ca:P ratio of 1.33. At 21 and 42 days of life, birds were killed, the crop, ileum, and caeca were longitudinally opened, and digesta was obtained with a sterile spoon. Samples were pooled on a pen basis and immediately stored at -80°C until further analysis. DNA of 296 samples was extracted with a commercial kit and 16S rRNA gene target sequencing was performed, followed by bioinformatic analysis with Mothur [1] and PERMANOVA routine was used to study the significant differences and interactions between treatments.

Results: Permanova showedsignificant microbiota differences between the species, time point and the gastrointestinal section ($p < 0.05$). Moreover, significant CaP level effects were detected in turkeys at both time points in ileum samples and at 21 days in the caeca ($p < 0.05$). Significant phytase effects were observed in the caeca of turkeys and broilers at 42 days ($p < 0.05$). Abundance levels of Ligilactobacillus, Faecalibacterium and Romboutsia in the crop of turkeys were higher than in broilers ($p<0.05$). Ligilactobacillus and Peptostreptococcaceae were more abundant in the ileum of turkeys than broilers. The caeca samples were more similar between the species, and the sampling time point significantly affected the data for this gastrointestinal section. Correlations of performance traits such as average daily gain and taxonomy data showed significant interactions on genera depending on the species, age, CaP, and phytase. In the ileum of 42-day-old turkeys fed with the CaP- diet, the myo-inositol concentration was negatively correlated to Ruminococcaceae, and $InsP_6$ concentration was negatively correlated to Lachnospiraceae. In the caeca of 42-day-old turkeys, the $InsP_6$ concentration in Phy- was negatively correlated to Phocaeicola and positively correlated to Anaerobutyricum in Phy+. Further, the myo-inositol concentration in the caeca of 42-day-old broilers with Phy+ was positively correlated to Corynebacterium, whereas a negative correlation of the measured myo-inositol concentration to Ligilactobacillus was found in Phy-.

Conclusions: Under the same feeding and housing conditions, we observed distinct microbial assemblages across the gastrointestinal tract of broilers and turkeys of the same age. Diets did not affect microbial assemblages. Correlations of the microbial composition with performance data suggest cross-talk between microbiota and nutrition, which merits further investigations.

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26.

Effects of two probiotic Bacillus-strains on PBMCs of broiler chicken

Effekte zweier probiotischer Bacillus-Stämme auf PBMCs von Broilern

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Probiotics as feed additives have the potential to support animal health and, thus, may help to reduce the usage of antimicrobials in poultry production. Bacillus subtilis DSM 32315 (BS) and B. amyloliquefaciens CECT 5940 (BA), two commercially available probiotics, have been described to provide a health benefit which is attributed to the modulation of gut microbiome, nutrient uptake, gut integrity, and the immune system [1-2]. However, the underlying mechanisms of probiotic feed additives are still elusive as they may directly influence immune cells or the intestinal milieu. Here, we investigated the direct immunomodulatory effects of BS and BA using a chicken immune cell assay with peripheral blood mononuclear cells (PBMCs), a useful *in vitro* model based on primary cultured chicken immune cells, which more closely mimic the *in vivo* conditions than conventional single cell lines.

Methods: PBMCs were isolated from blood of Cobb500 broiler chicken. Animals were fed the same starter diet post hatch from day 1 to 14 and the same grower diet afterwards (H. Wilhelm Schaumann GmbH). For co-culture experiments, $1x10^6$ PBMCs were treated with either vital BS or BA in a ratio of 1:3 (PBMCs:Bacillus) for 24 hours in RPMI 1640 medium supplemented with 10 % chicken serum at 41 °C with 5 % CO_2. Concanavalin A (conA, 10 µg/ml) was used as a positive control. Changes in the composition and activation status of PBMCs were monitored via flow cytometry (BD FACSCanto II, BD Life Sciences). Using monoclonal antibodies, CD4+ T-helper cells (CD4-PE), CD8+ cytotoxic T-cells (CD8-APC), CD25+ activated T cells (CD25-FITC) and Bu-1+ B cells (Bu-1a-FITC) were phenotyped. Non-viable cells were excluded during gating using DAPI as a viability marker. Analysis was performed using FlowJo™ v10.5.0 Software (BD Life Sciences). The cell count measured relative to the vital PBMC population was visualized as the difference Δ between the treatment and the control for every biological replicate. Statistical significance was evaluated using a one-sided Student's t-test against a hypothetical value "0".

Results: We detected a higher Δ relative cell count of CD4+ T-helper cells ($p < 0.05$, N=11) and CD4+CD25+ activated T-helper cells ($p < 0.01$, N=11) in PBMCs treated with BS. Moreover, the Δ relative cell count of CD8+ cytotoxic T cells ($p < 0.05$, N=13) and CD8+CD25+ activated cytotoxic T cells ($p < 0.05$, N=13) was increased after treatment with BS. Bu-1+ B cells remained unaffected by BS (N=6). For BA treated PBMCs, we measured an increased Δ relative cell count of CD4+ T-helper cells ($p < 0.1$, N=11) and CD4+CD25+ activated T-helper cells ($p < 0.05$, N=11) like we observed with BS-treatment. However, the Δ relative cell count of CD8+ cytotoxic T cells (N=11), CD8+CD25+ activated cytotoxic T cells (N=11) and Bu-1+ B cells (N=6) did not change after treatment with BA.

Conclusions: Our results suggest an immune-modulating effect of both Bacillus strains on PBMCs. By the investigation of immunomodulatory effects of probiotics and contribution to the understanding of the underlying molecular mechanisms, our study may help to reduce and prevent the usage of antimicrobials in farming.

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27.

The impacts of a Bacillus-based probiotic on broiler chickens fed with increasing dietary levels of rye

Die Auswirkungen eines Probiotikums auf Bacillus-Basis auf Broilers, die mit zunehmendem Roggenanteil im Futter gefüttert werden

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The current study evaluated the effects of different inclusion levels of a 3-strain Bacillus-based probiotic (BP, a B. amyloliquefaciens and two B. subtillis strains) in diets with different concentration of rye on growth performance and nutrient digestibility of broilers.

Methods: Nine isocaloric and isonitrogenous experimental diets were produced by adding 0, 1.2 x 10^6 and 1.2 x 10^7CFU/g feed of BP to broiler diets containing 0, 20% and 40% of rye. In total, 2,160 one-day-old broiler chicks were randomly allocated to 216 pens and the diets were randomly assigned to the pens. Growth performance variables were recorded weekly and the apparent ileal digestibility (AID) of nutrients was measured at d 35. Data were subjected to ANOVA using the GLM procedure.

Results: Inclusion of BP in diets had no impact on performance variables, ileal and faecal viscosity as well as ileal flow of galactosamine (as a marker for mucin) and footpad dermatitis (FPD) score ($P > 0.05$). Birds feeding diets with 40% rye showed the worst body weight gain and feed conversion ratio (FCR) during the first 7 and 21 d of age. At the end of the trial, FCR of birds feeding diets with 40% rye was higher than birds receiving diets with no rye ($P \leq 0.05$). Increasing in rye concentration in diets, increased ileal viscosity (0% < 20% < 40%) and decreased (0% > 20% > 40%) AID of crude protein, P and all the amino acids. Broilers receiving diets with 40% rye showed higher faecal viscosity and lower AID of fat and Ca compared with other two groups ($P \leq 0.05$). However, adding rye to the diets (20% and 40%) led to higher FPD score for broilers and higher ileal flow of mucin (mucus loss) compared with broilers feeding diets with no rye ($P \leq 0.05$). Adding the BP product to diets impaired AID of P, while birds receiving diets containing highest concentration of BP showed highest AID of Met and Cys ($P \leq 0.05$).

Conclusions: In conclusion,adding rye to broilers diets increased digesta viscosity which led to higher rate of mucus loss, more incident of FPD, lower nutrients digestibility and finally worse growth performance. However, adding high level of a 3-strain Bacillus-based probiotic to broilers diets could improve AID of Met and Cys.

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28.

First data on the effects of different feed additives with potential ability for ammonia fixation in excrements on the zootechnical performance of slow-growing broiler chickens

Erste Daten zu den Auswirkungen verschiedener Futterzusatzstoffe mit potenzieller Fähigkeit zur Ammoniakfixierung in den Exkrementen auf die zootechnische Leistung von langsam wachsenden Masthühnern

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European organic broiler production comprises excessive nitrogen concentrations in feed since low-protein strategies by applying crystalline amino acids and exogenous enzymes are prohibited. Excess dietary nitrogen can increase microbial ammonia formation in the hindgut and excretments, respectively, which increases the metabolic burden for the bird and impairs the quality of stable air [1]. We tested a variety of feed additives, which are promoted either to bind excess ammonia in the gut due to their higher relative surface area (Klinofeed, Bentonit, and BioChar) or to increase ammonia fixation in microbial protein by supplying fermentable fiber (Arbocel). In a first step, we report the zootechnical performance monitored during the first experimental run.

Methods: 120 slow-growing mix-sexed ROSS broilers were fed the same diet (FORS 2121; 13 MJ ME/kg, 200 g/kg CP) during a 10d starter period and subsequent 5 weeks of fattening. This one-phase feeding regime was applied to ensure excessive nitrogen intake during all steps of the experiment. Birds were housed in five pens of 24 animals each during the starter period (each comprising one feeding group) and pair-wise in metabolic cages during fattening. Distribution of birds over cages was done randomly (n = 12 cages or 24 birds per group, respectively). The following dietary treatments were assigned: Control, 5 g/kg Klinofeed (source of clinoptilolite), 5 g/kg Bentonit, 5 g/kg BioChar (source of carbon and ashes), 6 g/kg Arbocel. Data collection comprised cage-wise feed intake weekly, bird-wise life weight at the end of starter and fattening phase, respectively, as well as carcass weight. Data was subjected to mixed model or repeated measures mixed model analysis depending on the type of data and in each case comprising either the bird or cage nested under the respective feeding group as random variable.

Results: Klinofeed birds entered the fattening phase with lower life weight (291 g/bird) compared to all other groups (313, 324, 323, 315 g/bird in Control, Bentonit, BioChar, Arbocel; $P < 0.0001$, no interaction with sex). However, at the end of fattening no relevant difference in live weight (2735, 2673, 2744, 2755, 2697 g/bird in Control, Klinofeed, Bentonit, BioChar, Arbocel) or carcass weight (2004, 1938, 2000, 2014, 1963 g/bird in Control, Klinofeed, Bentonit, BioChar, Arbocel) between groups was evident ($P = 0.63$ and 0.50, respectively; no interaction with sex). Klinofeed birds even showed numerically decreased total feed intake over the whole study period (7951 g compared to 8566 g, 8414 g, 8472 g, 8163 g in Control, Bentonit, BioChar, Arbocel; $P = 0.08$) and, accordingly, improved feed:gain (3.21 compared to 3.36, 3.36, 3.28, 3.34 in Control, Bentonit, BioChar, Arbocel; $P = 0.49$), when observed over the complete fattening period.

Conclusions: Although Klinofeed apparently exerted some antinutritive effects during the starter phase, these birds fully compensated their initial losses during fattening, growing to a weight comparable to all other groups including Control. Interestingly, despite the fact that all tested additives diluted the diets in terms of metabolizable DM (~0.3 MJ ME/kg less compared to control) no relevant performance differences compared to control were evident in the end of the study. This suggests that these additives all exerted some positive effects on the animals that either improved feed utilization or saved the birds some energy and nutrients endogenously. This is currently studied in more detail.

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29.

Effects of a wood-derived feed supplement on laying persistency and egg shellstability of laying hens throughout a late production period

Einfluss eines holzbasierten Futterzusatzes auf die Legepersistenz und Eischalenstabilität von Legehennen in einer späten Produktionsperiode

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The goal of a prolonged production cycle in high-performing laying hens requires both the maintenance of a high laying persistency as well as the stabilization of eggshell quality: with increasing age not only the absolute thickness of the eggshell declines, also a long-lasting stress exposure during weeks of high laying rates will affect formation of matrix proteins resulting in an increased breaking rate. This increase in breaking rate is of economic relevance and appears to be a limiting factor in the prolonged and, thus, sustainable use of laying hens. The objective of the present study was to determine the effects of a supplementation of a wood-derived complementary feed based on a bark, rich in lignans, (agromed®ROI, agromed Austria GmbH) on the performance of commercial laying hens during the late stage of their production cycle as well as on eggshell stability of aged hens. Due to the described anti-inflammatory properties of the wood derived product [1], a beneficial impact on zootechnical performance and eggshell breaking strength of layers in a late production period was hypothesized.

Methods: 90 healthy laying hens (Lohmann Brown) at the age of 61 weeks were allocated equally according to body weight and laying rate to 30 pens. Each pen was assigned to one of three treatments, resulting in 10 repetitions á 3 birds per treatment. Birds of the control group were fed on a standard diet based on corn and soybean meal, whereas diets for experimental groups were supplemented with the wood-derived product in a dosage of 200 ppm and 400 ppm, respectively. Diets were formulated to be isoenergetic and isonitrogenous. Throughout a trial duration of 112 days, body weight change, feed intake, egg mass and egg weight as well as eggshell stability of 10 eggs per treatment were measured in 14-day intervals. Data were analysed by performing One-Way-Anova according to completely randomized design using the software package SPSS (IBM SPSS Version 25). Multiple comparisons between treatment groups were made by Tukey's test. Significant differences were considered at $p \leq 0.05$, while near significant trends were considered for $0.05 < p \leq 0.10$.

Results: Throughout the complete trial duration the wood-derived supplement did not markedly affect body weight change. Although the diets were formulated to be isoenergetic hens fed the feed supplement consumed on average significantly less feed than birds of the control group (-3.9%). Comparisons between dose levels showed that feed intake deceased with increasing dose level. The wood-derived feed supplement at an inclusion rate of 400 ppm did significantly enhance overall egg mass output by 2.8% ($p=0.015$) and laying rate by 2.1%($p=0.018$) compared to the control group; even not significant comparisons between dose levels showed dose dependent effects. As a result of the significantly reduced feed intake in combination with the enhanced laying performance, the feed-to-egg mass ratio was significantly reduced in both treatments 200 and 400ppm (2.094 and 1.983, resp.) compared to the control group (2.166; $p<0.001$). There were no consistent differences among treatment groups in eggweight, egg yolk and albumen characteristics. However, the averaged eggshell breaking strength in birds supplemented with a dose of 400 ppm of the feed supplement tended to be significantly higher than that found in the control group (+5.1%; $p=0.077$); improvements were less with inclusion 200 ppm (+2.6%).

Conclusions: A dose responsive effect of the feed supplement was demonstrated regarding both, laying performance and eggshell stability. Although the wood-lignans in the product are described to be antioxidative, the product's impact on the oxidative status of the hens turned out to be moderate only. Thus, our conclusion, that the anti-inflammatory mode of action contributes to a facilitated nutrient uptake and affects the formation of eggshell matrix proteins, needs to be further investigated.

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30.

Impact of essential oils on the composition and activity of porcine ileal and faecal microbiota – an *in vitro* batch fermentation assay

Einfluss ätherischer Öle auf die Komposition und Aktivität der ilealen und fäkalen Mikrobiota von Schweinen – ein in vitro Batch Fermentationsversuch

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In recent years, the interest in essential oils as feed additives for pigs is steadily increasing. Among them, the phenylpropenes eugenol (e.g. in cloves and cinnamon) and cinnamaldehyde (e.g. in cinnamon) as well as the monoterpene menthol (e.g. in peppermint) are commonly used. Besides other health promoting effects, these essential oils are known for their antimicrobial activity [1, 2]. Microbial fermentation processes can be investigated via static batch fermentation systems [3]. Thus, the aim of this study was to investigate the influence of cinnamon (CIN), clove (CLO) and peppermint (PEP) leaf oils on the fermentation process of ileal and faecal microbiota in a batch fermentation model. In addition, selected metabolites (SCFA, ammonium) as well as the microbial composition were examined.

Methods: Samples of ileal digesta and rectal contents have been taken from six 12-week-old pigs (m/w, DanBred x Duroc) and kept frozen at -20°C. In 6 replicates, the ileal and faecal samples were thawed, and batches were merged with inoculum (10 % w/v), three essential oils and a PRAS (pre-reduced anaerobically sterilized) buffer enriched with 5 g/L peptone. The 11 treatment groups (CIN, CLO, and PEP at 10/5/0.1 and 0.01 mg/g inoculum, respectively) were incubated anaerobically for 24 h at 37°C. A control treatment without oil at 0 h and 24 h was provided. Subsequently, the batches were centrifuged, and the bacterial pellet was stored frozen at -80°C. The pH was measured in the supernatant before aliquoting and freezing it for metabolite analysis. SCFA were analyzed by gas chromatography and ammonium by Berthelot reaction. After extracting the DNA from the bacterial pellets using a commercial kit, a 16S-rDNA sequencing of all the samples except those treated with oils at 5 or 0.1 mg/g inoculum was conducted. The data analysis was performed using Kruskal-Wallis- and Mann-Whitney-U-Test where applicable (R, version 4.2.1, significance at $p \leq 0.05$). For each product, a PCA was performed via ClustVis taking the 25 most abundant genera but also phyla, Shannon-Index (ASV), SCFA and ammonium into account.

Results: The PCA demonstrated that for each of the three products, the variables that have been considered for the samples treated with 10 mg/g inoculum of CIN/CLO or PEP form a cluster. Regarding the microbial diversity indices, significant differences could be seen for the products CIN, CLO and PEP at a concentration of 10 mg/g inoculum (either faecal or ileal) compared to the untreated control. Specifically, in ileal microbiota, CIN and CLO decreased the Shannon-index, the richness index was moreover decreased by CLO and PEP at this concentration. Regarding the faecal microbiota, the richness index was reduced by all the three products and PEP further decreased the Shannon-index.

Conclusions: The impact of the three tested essential oils on the microbial abundance and activity in ileal digesta and faeces is concentration dependent. Essential oils at a concentration of 10 mg/g each, seem to reduce the microbial diversity in ileal digesta and faeces. The effect of the essential oils seems to be comparable on ileal and faecal microbiota.

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31.

Utilization of anaerobic fungi culture supernatant as a novel silage additive for drought-impaired grass

Nutzung des Überstands anaerober Pilzkulturen als neuen Silierzusatz für dürregeschädigtes Gras

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Pronounced droughts periods have become more frequent in Europe causing water stress in grassland, which is expected to impair the ensiling properties of grasses [1] and so the supply of ruminants with good quality forages. Additional treatments of forages may allow a higher exploitation of such substrates by pre-cleaving fiber structures. Anaerobic fungi (AF) possess a diverse enzyme repertoire and thus are efficient degraders of tough plant fiber [2]. During AF cultivation, substantial amounts of enzymes are released into the culture medium, the supernatant of which was recently shown to enhance the ruminal cellulose degradability of grass silages by disrupting lignocellulosic biomass in the silo [3]. To assess the suitability of this silage additive type for drought-impaired plant material, our study investigated the impact of adding a new AF supernatant on the quality and *in vitro* gas production kinetics of silages prepared from drought-impaired grass. We hypothesized a higher gas production for silages treated with AF supernatant than for controls.

Methods: The AF supernatant containing the AF enzymes was obtained from the fungal strain Feramyces DF1, isolated from deer faeces. Therefore, the complete fungal culture was centrifuged and the supernatant was stored at -20 °C until ensiling. The silages were produced with approximately 35% dry matter (DM) concentration in vacuum plastic bags using hay from a grass stand (93.4% grasses, 3.2% legumes, 3.4% herbs) that was produced under controlled drought conditions at the Agricultural Research and Education Centre Raumberg-Gumpenstein (Irdning-Donnersbachtal, Austria). Regarding the applied treatments, grass hay was ensiled with i) fresh AF supernatant (25% of ensiled DM), ii) heat-inactivated AF supernatant as control (25% of ensiled DM), or iii) without an additive as a negative control. Each treatment was prepared in quadruplicate and stored for 90 days. Subsequently, the proximate nutrient composition and fermentation patterns of all silages were determined and the data was analyzed using the GLM procedure of SAS v9.4 (SAS Institute Inc., USA), while the *in vitro* gas production kinetics are currently in analysis.

Results: All silages may be classified as well fermented as pH was below 3.9 without differences between treatments ($P=0.08$) and butyric acid was not detected. Similarly, acetic acid concentrations were on an overall low level with 0.54 vs. 0.30 and 0.39% of DM for grass silage with fresh AF supernatant, heat-inactivated AF supernatant and without an additive, respectively; the latter two values being lower than the first ($P<0.01$). However, the DM losses were higher in grass silages produced with heat-inactivated AF supernatant than in grass silages produced with fresh AF supernatant or without an additive, i.e. 5.8 vs. 3.6 vs. 3.6%, respectively ($P<0.01$). Regarding the fiber composition, the hemicellulose fraction was reduced by approximately 6 percentage units when using the fresh AF supernatant ($P<0.01$) and therefore substantially lower when compared to the silages prepared with heat-inactivated AF supernatant or without an additive (14.1 vs. 20.1 vs. 20.3% of DM, respectively). Likewise, the cellulose content was lower with fresh AF supernatant (27.0% of DM) than with heat-inactivated AF supernatant (30.8% of DM; $P<0.05$), whereas it was only numerically lower than in grass silage without an additive (28.7% of DM; $P>0.05$).

Conclusions: Our pending analyses will reveal whether the beneficial effects of fresh AF supernatant observed at silage composition level can result in improved *in vitro* gas production kinetics. However, the present data suggest that the enzymes present in AF supernatant can pre-cleave fibrous structures during ensiling and therefore potentially promote the energy exploitation from drought-impaired grass by the ruminant.

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32.

Determination of non-edible biomass abundance in Germany‘s agricultural and processing sector

Ermittlung des Aufkommens nicht essbarer Biomasse aus dem Agrar- und Verarbeitungssektor Deutschlands

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The role of livestock can be defined as extractors and concentrators of nutrients from non-edible biomass to human-edible foods [1]. While this role is still true today, high concentrate and low roughage amounts in livestock rations increasingly contribute to a food and feed competition between humans and animals. Using non-edible plant biomass efficiently by refining it via livestock can create high quality food products without contributing to this competition. Since non-edible biomass is inevitably coupled to edible biomass production, the role of livestock will also be important in future agricultural systems. The aim of this study was to assess the amount of plant biomass produced annually in germany and its distinction into human edible biomass **[eB]** and non-edible biomass **[neB]** .

Methods: Literature research has been conducted (26 peer reviewed publications, 15 books, 26 reports, 15 other grey literature) to identify the amount of **eB** and **neB** along the agricultural production chain. Therefore, conversion efficiencies of biomass from field to fork have been calculated on a dry matter basis by considering main- to by-product ratios on the field as well as processing efficiencies for the most important food and renewable energy industry branches (e.g., milling, ethanol production, brewing, oil extraction, starch extraction, sugar extraction). Based upon these calculations, the human edible fraction (HeF) [2][3] was used together with Germany’s mean agricultural production data during the years 2012-17 to determine the abundance of **eB** and **neB** in Germany’s agricultural and processing sector for two scenarios: low vs. high edibility of biomass. Since HeF factors denote theoretical margins of edibility of biomass, additional scenarios were calculated assuming current processing efficiencies of the food and renewable energy industry as well as silage maize being completely inedible. Furthermore, the changes in biomass production for a complete substitution of land used for silo maize production and cultivation of human edible foods (cultivation structure 2012-17) have been analysed.

Results: Calculations for the two HeF scenarios showed, that 115-134 million tonnes of **neB** and 38-57 million tonnes of **eB** were produced annually during the observation period. This equals a theoretical margin of 2-3.5kg of **neB** per kg of eB. When maize that is used for silage was considered as non-edible, the numbers increased to 2.5-4.8kg of **neB** per kg of **eB**. When Germany’s current processing efficiencies were used for calculations instead of the two HeF scenarios, and silage maize was considered not edible, then 1kg of **eB** produced 4kg of **neB**. If the area used for silage maize production would in future only be used to produce human edible field crops (cultivation structure 2012-17) and current processing efficiencies were assumed, then 1kg of eB yielded 2.8kg of **neB**.

Conclusions: The research shows that the amount of edible biomass per acre of agricultural land changes dramatically once the whole agricultural system including grassland, green plants for feed production, straw, and by-products is part of the calculations. Due to carbon sequestering potentials of grassland and the also limited withdrawal of straw per acre of land in food production the amount of human edible proportion of biomass is mainly influenced by processing efficiencies and by maximizing the amount of extracted edible biomass from the total biomass harvested. Even substituting the area used for green plants for feed production such as silo maize with food crops such as wheat, barley etc. will always produce significant amounts of non-edible biomass that can be efficiently refined to high quality food products by animals. The future challenge will be to improve current processing efficiencies of the food and renewable energy industry in order to approach the upper limits of edibility given by the HeF fractions.

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33.

Effect of naturally occurring heat stress on dry matter intake, apparent digestibility of organic matter and milk production in dairy cows: Does the addition of biochar attenuate the consequences?

Einfluss von unter natürlichen Bedingungen auftretendem Hitzestress auf Trockensubstanzaufnahme, Verdaulichkeit der organischen Substanz und Milchproduktion von Milchkühen: Verringert das Verfüttern von Pflanzenkohle die Folgen?

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While the average number of days with temperatures above 30°C amounted to 8.7 between 1961 to 1990 it increased to 16.3 during the following 30 years [1]. Decreased dry matter intake (DMI) and milk yield (MY) are well known consequences of heat stress (HS) in dairy cows. Recent studies also indicate an increase in gut-permeability [2], which could lead to a higher transfer of microbiological metabolites and thus a negative impact on health. Biochar is already used in cattle feeding. Due to its potential to adsorb toxins, it might ameliorate the negative impact of HS and exert an effect on performance. The aim of the present study was to investigate the influence of HS and the use of biochar as a feed additive on DMI, apparent digestibility of organic matter (ADOM) and MY in Holstein dairy cows under naturally occurring HS in Germany.

Methods: Between July and September 2022, 18 dairy cows kept in the same barn were allocated to two groups stratified for parity, MY and days in milk. Biochar (Carbuna TFK) was added to the total mixed ration (0.7% of DM) in a crossover design (4 wk of experimental feeding, 2 wk of washout in between). Individual DMI was continuously measured. Temperature and humidity in the barn were recorded every 15 min. MY was documented daily, milk quality testing was done weekly. For a subgroup of 5 cows per group, three spot samples of faeces were collected during a period of 12 h once a week. ADOM was calculated using acid-insoluble ash as internal marker. Respiratory rate (RR) and rectal temperature (RT) were recorded for 10 animals at 5 p.m. on Thursdays and 7 a.m. on Fridays. In addition, this clinical scoring was carried out for all animals between 2 p.m. and 3 p.m on the warmest day of every wk. The Temperature Humidity Index (THI) was calculated for every timeslot as suggested by the German Agricultural Society [3] and the area under the curve for times with a THI above 68 was added to the cumulative THI (cTHI) for each day. Energy Balance (EB) was calculated based on DMI, MY and body weight for every animal. Scoring data was analysed by linear regression. Effects on DMI, EB, ADOM and MY were analysed using a generalised mixed linear model with cow as random effect, day as repeated measure and cTHI and diet as fixed factors. Statistical analysis was carried out using SPSS 27, IBM.

Results: When THI did not exceed 68 during the clinical scoring, the animals presented with physiological RR (48 ± 12 breaths per min) and RT (38.5 ± 0.39°C) and correlations with THI were moderate (Spearman-Rho correlation coefficients (r): 0.219 for RR and 0.371 for RT). When THI was increased to values ≥ 68, RR rised by 2.21 breaths per min (R^2: 0.409) and RT by 0.09 K (R^2: 0.496) with every THI unit. The cTHI representing HS during the entire day exerted a negative effect on DMI on the same day ($P<0.01$) and on MY two days later ($P<0.01$). A cTHI value above 480 that was determined on 14 out of 70 d and is equivalent to moderate HS (THI: 73) for 24 h or a greater degree of HS during less time, reduced the DMI by 1.98 kg, the EB by 15.51 MJ NEL and the MY two days later by 0.7 kg in comparison to days with less HS. Addition of biochar slightly decreased DMI (0.375 kg DM, $P<0.05$) and EB (4.01 MJ NEL, $P<0.01$) but did not affect MY. The preliminary results of the analysis of ADOM during the entire observation period amounted to 69.5% and varied over time. However, no clear effects could be found for either cTHI or biochar.

Conclusions: From the acute effects of THI on RR and RT it can be concluded that HS in the German summer can exceed the adaptability of dairy cattle and lead to tachypnoea and hyperthermia. The reduction in energy intake is more pronounced than the decrease in MY and can induce or exacerbate a negative EB. Adding biochar cannot prevent the decrease in performance but might slightly reduce DMI. Further data analysis should include the influence of HS on feeding behaviour and resilience of different individuals to these challenges.

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34.

Mild heat stress is associated with immunological and metabolic changes in peripheral blood mononuclear cells of primiparous Holstein dairy cows

Milder Hitzestress ist assoziiert mit immunologischen und metabolen Änderungen in peripheren mononukleären Blutzelle von primiparen Holstein Kühen

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High ambient temperatures and humidity are significant environmental stressors compromising animal health, along with a reduction in feed intake, milk production and alterations of the immune system. Peripheral blood mononuclear cells (PBMC) are composed of monocytes, natural killer cells, B and T cells, all involved in various immunological functions. Heat stress induces changes in this immune cell composition modulating immunoglobulin and cytokine production [1]. However, the exact metabolic and immunological response of PBMC during heat stress and reduced nutrient intake has been scarcely explored. Therefore, our objective was to elucidate possibly occurring immune modulatory responses and metabolic signaling pathways of PBMC after six days of heat stress compared to cows kept at thermoneutrality.

Methods: Twenty primiparous, non-pregnant German Holstein cows (169±9 DIM) were evenly allocated to either a heat-stressed (HS, n=10), control (CON, n=10) and pair-fed (PF, n=10) group. During adaptation phase, all animals were kept in a climate chamber at conditions defined earlier to be thermoneutral (TN) for lactating dairy cows [2,3]: 16°C, 63% relative humidity (RH), temperature-humidity index (THI) 60 for 6 days (d) and received a total mixed ration twice daily. In the experimental phase, HS cows were exposed to 28°C for 6 d (52% RH, THI 76) with ad libitum feeding. CON and PF groups were kept under TN conditions for 6 d. CON cows had ad libitum access to feed; PF cows received the same amount of feed as HS cows. Dry matter intake per kg body weight (DMI/BW), milk yield, rectal temperature (RT), and respiration frequency (RF) were measured daily. Blood samples were taken after 6 d and PBMC were isolated by Histopaque gradient. RNA was isolated and stranded RNAseq libraries were prepared and run in a multiplex-design with 2 x 100 bp paired-end sequencing cycles on the Illumina HiSeq2500 system. Data were mapped to the bovine reference genome (ARS-UCD1.2 and Ensembl Annotation; Release 95) with HISAT2. Count matrices for gene expression were established by the featureCounts option of the subRead package. Differential expression analysis between the two experimental groups was performed with DESeq2 and with Benjamini-Hochberg adjustment to account for multiple testing. The differentially expressed genes with $p_{adj} < 0.05$ were further analyzed by DAVID, KEGG and ClueGo for pathway-enrichment analysis.

Results: From d 2 to 6, DMI/BW was lower in HS and PF than CON cows ($P<0.001$). On d 6, milk yield was lower in HS than PF and CON cows ($P<0.05$). From d 1 to 6, RT and RF were higher in HS than PF and CON cows ($P<0.001$). In total, 315 genes were differentially expressed ($q<0.05$), 65 genes were lower and 250 gene were higher expressed in HS than CON cows. In contrast, 501 genes were higher and 378 genes were lower abundant in HS than PF cows ($q<0.05$). The functional pathway analysis in DAVID showed an enrichment of a predominant number of higher expressed genes in platelet activation, regulation of actin cytoskeleton, leukocyte transendothelial migration, tight junction, focal adhesion and hematopoietic cell lineage in HS than CON cows. Furthermore, the analysis with ClueGo highlighted that immunological processes were activated for megakaryocyte differentiation, positive regulation of leukocytes chemotaxis and erythrocyte differentiation in HS than CON cows. However, protein lipidation, cellular amino acid metabolic processes, lipid and fatty acid oxidation were downregulated in CON than in HS cows.

Conclusions: Our data suggest that PBMC are affected by mild heat stress inducing leukocyte chemotaxis and migration, blood coagulation, anchoring cell junction, protein stabilization and changes in nutrient utilization to maintain immunohomeostasis. If the reduction in energy and nutrient intake under high ambient temperatures accounts for the changes in genes expression remains to be investigated in future studies.

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35.

Mild heat stress increases ruminal acetate synthesis and alters milk composition of primiparous Holstein dairy cows

Milder Hitzestress führt zur Erhöhung des Anteils der ruminalen Acetatproduktion und beeinflusst die Milchzusammensetzung von primiparen Holstein Kühen

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Ambient temperatures have considerable influence on dairy cows causing a reduction in feed intake and milk yield. Feed intake and diet composition are the main factors influencing ruminal short-chain fatty acid (SCFA) concentrations, the latter in turn may influence milk composition. However, the link between SCFA production and milk composition during heat stress has been less investigated. Therefore, the objective of this study was to elucidate milk composition and ruminal SCFA production after six days of heat stress relative to pair-fed dairy cows kept at thermoneutrality.

Methods: Thirty primiparous, non-pregnant German Holstein cows (169±9 days in milk) were evenly allocated to three groups: heat-stressed (HS, n=10), control (CON, n=10) or pair-feeding (PF, n=10). For the first 6 days (d) of adaptation, all animals were maintained at thermoneutal [1,2] conditions (TN, 16°C, 63% relative humidity, temperature-humidity index (THI) = 60) in a climate chamber and received a total mixed ration twice daily (0730 and 1730 h). In the subsequent experimental phase, HS cows were exposed to 28°C for 6 d (THI = 76) with ad libitum feeding and access to water, both tempered to 28°C. The CON group was exposed to 16°C (THI = 60) with ad libitum feeding for 6 d. The PF cows were exposed to the same environmental conditions as CON cows, but were offered only the amount of feed the HS cows ingested. Milk yield and dry matter intake (DMI) were measured daily. Milk composition and blood samples were analysed in the adaptation phase, on d 3 and 6 of the experimental period. Rumen fluid samples were taken in parallel by an oral rumen tubing method. Ruminal pH was determined and SCFA concentrations were measured by a gas chromatograph equipped with a flame ionization detector and calculated as percentage of the total (mol/mol). Surface temperature of the rumen was measured by infrared thermography on d -1 and d 6 of the experimental phase. Daily measurements on the same animal were analyzed with a repeated measure ANOVA using the MIXED procedure of SAS (Version 9.4) with the fixed effects of treatment (HS, CON, PF), time (d), and treatment by time interaction; and days in milk served as a covariate. Multiple comparisons were tested with a Tukey-Kramer test.

Results: During the adaptation phase, groups did not differ in DMI, milk yield, milk composition, and SCFA concentrations. Surface temperature of the rumen was higher under HS than TN conditions ($P<0.001$). From d 2 to 6 of the experimental phase, DMI of HS and PF was lower than in CON cows ($P<0.001$). From d 3 to 6, milk yield was lower in HS than CON cows ($P<0.05$). Milk fat yield was lower in HS than PF cows on d 6, whereas milk protein yield and lactose yield were lower in HS than CON on d 3 and 6. On d 6, milk protein yield was lower in HS than PF cows ($P<0.05$), whilst protein, fat and lactose percentage were unaltered among the groups. On d 6, milk urea and plasma urea concentrations were higher in HS than CON and PF cows ($P<0.05$). On d 6, energy corrected milk yield was lower in HS than CON and PF cows ($P<0.05$). Ruminal pH and total ruminal SCFA concentrations were comparable between groups. The portion of acetate and the acetate:propionate ratio were greater in HS than CON cows on d 3 and 6 ($P<0.05$), and were higher in HS than PF cows on d 3 ($P<0.05$). On d 6, n-valerate was lower in HS and PF than CON cows ($P<0.05$, respectively), whilst butyrate was not different between groups.

Conclusions: Our data suggests that heat stress affects ruminal fermentation independent of the reduction of DMI by favoring ruminal acetate over propionate synthesis. However, we cannot exclude reduced ruminal acetate absorption, which might explain lower milk fat yield of HS cows. Furthermore, it seems that heat stress reduced microbial protein synthesis and increased endogenous protein catabolism as indicated by the lower milk protein yield, higher milk and plasma urea concentrations.

Acknowledgement: This study was founded by the DFG (KO 5972/2-1).

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36.

Effect of a blend of essential oils on ruminal fermentation characteristics, methane emission and milk yield in dairy cows fed a hay or a silage based diet

Wirkung einer Mischung ätherischer Öle auf die Pansenfermentation, Methanemission und die Milchleistung von mit Heu oder Silage gefütterten Milchkühen

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Various feeding strategies have been investigated in the past to reduce methane (CH_4) emissions from ruminants [1]. Feeding additives including essential oils have been shown to be effective both *in vitro* [2] and *in vivo* [3]. However, it has not yet been conclusively clarified whether the composition of the basal diet has an influence on the effect. The aim of the present study was, therefore, to investigate the effect of a blend of essential oils supplemented to a hay or a silage based diet on ruminal fermentation characteristics, CH_4 production, feed intake and milk yield of dairy cows.

Methods: Thirty-two lactating Holstein Friesian and Red Holstein cows (milk yield: 30.6±4.83 kg/d; days in milk: 79.4±31.3 d) were assigned to four treatment groups balanced by milk yield, days in milk and lactation number. The study was split into a pre-period (week 1-4), an adaptation period (week 5-8, AP), an experimental period (week 9-12, EP) and a post-period (week 13-16). During the whole study, half of the cows (n=16) were fed hay (H; NEL, 6.1 MJ/kg dry matter (DM); crude protein (CP), 153 g/kg DM), the other half (n=16) fed a silage based diet (S; NEL, 6.28 MJ/kg DM; CP, 118 g/kg DM) consisting of maize silage, grass silage and hay (44:43:13%). All cows were supplemented with a protein concentrate and an energy concentrate (50% maize, 30% barley, 20% wheat) to meet the requirements. They also got 300 g/d of a mineral feed. In AP and EP, for half of the H cows (n=8) and half of the S cows (n=8), 0.34% of the maize in the energy concentrate was replaced by a feed additive (Xtract Ruminant, Pancosma, Rolle, Switzerland) containing a blend of the essential oils, eugenol, cinnamaldehyde and capsicum. Milk yield and feed intake were recorded daily; milk components and diet composition were analysed weekly. Ruminal fluid was collected in the last week of each period using a stomach tube and analysed for ammonia and volatile fatty acids (VFA). The individual CH_4 production was determined using the GreenFeed© system (C-lock Technology Inc., Rapid City, SD, USA). Linear mixed models were used for statistical analyses with the fixed factors basal diet, feed additive, period and the random factor cow (R, package lme4).

Results: Cows receiving the feed additive consumed 1.00±0.05 g/d of it in AP and EP. The feed additive had no effect on the measured variables ($p>0.05$). Across all periods, the H cows fed more concentrate compared to S cows (4.89 kg vs. 3.83 kg/d, $p<0.05$). Intakes of total dry matter (DM) and crude protein did not differ (both $p>0.05$) between dietary treatments. TheHcows consumed more NDF (9.31 kg vs. 8.57 kg/d, $p<0.05$) and water-soluble carbohydrate (2.17 kg and 1.23 kg/d, $p<0.05$) compared to S cows. Daily milk yield, milk fat and protein percentages were numerically but not significantly (all $p>0.05$) lower for H compared to S cows. The H cows produced less energy corrected milk (ECM; 31.4 kg/d vs. 34.2 kg/d, $p<0.05$) than the S cows. The concentration of total ruminal VFA and the proportion of butyrate were lower (both $p<0.05$) and the proportion of acetate was higher ($p<0.05$) in H cows compared to S cows. Ruminal ammonia concentration differed between H and S cows ($p<0.05$). All cows produced similar amounts ($p>0.05$) of CH_4 per day (524 g) and CH_4 per kg DM intake (23.4 g). The CH_4 intensity (g/kg ECM) tended to be higher in H cows ($p=0.07$) than in S cows (17.0 vs 15.3 g/kg ECM). The production of CH_4 related to the NDF intake was less ($p<0.05$) in H cows compared to S cows.

Conclusions: Irrespective of the basal diet, the used blend of essential oils had no effect on milk yield. The missing effect on ruminal CH_4 production compared to other studies might be due to the use of other oils. The higher ECM yield in S cows resulted in a lower CH_4 intensity in silage compared to hay fed cows. Although H cows ingested more NDF, total CH_4 production was similar in all cows which consequently led to a lower CH_4 production per kg NDF intake in H cows compared to S cows.

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37.

Performance-based environmental impacts of substituting soybean meal by rapeseed meal in the diets for livestock

Leistungsbasierte Umweltwirkungen des Ersatzes von Sojaextraktionsschrot durch Rapsextraktionsschrot im Futter für Nutztiere

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Diets for growing pigs are commonly based on wheat and soybean meal (SBM). It is known that the highest emission of greenhouse gases comparing all protein sources comes from SBM mainly arising from land use change [1]. Rapeseed (extracted meal) is a regional protein source and has lower negative impacts on the environment ('CO_2 footprint'), compared to SBM [2]. In the production of livestock, feed production is known to be the main contributor to environmental impacts [3]. Commonly the method of life cycle assessment (LCA) is used to measure the environmental impacts of a product. Therefore, it was of interest to compare the environmental impact of compound feeds with increasing shares of RSM and moreover to determine the effects of substituting SBM by RSM in a diet on performance to have a comprehensive evaluation regarding effects on sustainability.

Methods: In consecutive trials 2 x 20 pigs (start of trial 1: age: 47 ± 0.489 days; bodyweight (bw): 15.1 ± 1.57 kg / start of trial 2: age: 50 ± 0.00 days; bw: 17.8 ± 2.86 kg) were housed individually in four feeding groups (group 1 – group 4) of five pigs each. Each group received a diet consisting of rye, SBM and/or RSM, barley, lignocellulose, soybean oil and further minor ingredients. The isonitrogenous diets were characterized by different shares (%) of both protein rich ingredients (SBM/RSM: group 1: 18.1/0; group 2: 13.6/6.70; group 3: 8.10/16.1; group 4: 0/28.0). Average daily feed intake (ADFI), gains (ADG) and the feed conversion ratio (FCR) were evaluated. An LCA of the individiual feedstuffs and the compound feeds were done following the ISO 14040 and 14044 and using the online software application Opteinics® (BASF, Lampertsheim) which is based on the Global Feed LCA Institute (GFLI) database. The impact on climate change is expressed in kg CO_2 equivalent (CO_2 eq), while 1 kg CO_2 eq equals the global warming potential of the emission of 1 kg CO_2 into the environment. In the first step, the assessment focused on the feed only. The system used the composition of the feed, the location of the feed mill and the energy usage of the feed mill. In the second step, the performance of the animals were taken into account. Statistics were done by SAS® Enterprise Guide® ($p < 0.05$).

Results: Substituting SBM by RSM in the compound feeds did neither influence the ADFI nor the ADG of the animals negatively. FCR differed significantly when the whole share of SBM was substituted by RSM (trial 1: group 1: 1.58a; group 4: 1,79b / trial 2: group 1: 1.62a; group 4: 1,79b). With rising amounts of RSM in the diets, the impact on climate change (impact of 1000 kg feed) of the diets decreased (diet I: 898.75 kg CO_2 eq, diet II 813.15 kg CO_2 eq, diet III: 725.99 kg CO_2 eq, diet IV: 601.09 kg CO_2 eq). Regarding the relative impact per ingredient (kg CO_2 eq/kg feedstuff), soybean oil had by far the highest value (7.34), followed by soybean meal (2.65) and quite low values for RSM (0.59). Focusing on the relative impact related to weight gain (CO_2 eq/kg weight gain), the highest mean values may be found with diet I (18.1 % SBM) for both trials (trial 1: 2.34a CO_2 eq/kg, trial 2: 2.33b kg CO_2 eq/kg). Although the highest FCR occurred in both trials, the significantly lowest relative impacts were reached with the feeding concept based only on RSM (trial I: 1.23a kg CO_2 eq/kg, trial 2: 1.32b CO_2 eq/kg).

Conclusions: Feeding diets containing increasing amounts of RSM to weaned piglets showed no negative effects on feed intake and gains of the animals. Even though, the FCR was negatively affected by really high amounts of RSM, a markedly lower CO_2 footprint was shown in the performance-based sustainability analyses for this feeding concept. Furthermore, the substitution of soybean oil should also be considered in terms of lowering environmental impacts. This sustainability assessment method seems to be appropriate for other livestock.

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38.

Contribution to reduced P-excretion: Option for feeding of pigs without supplementation of phosphates

Beitrag zur Reduzierung der P-Exkretion: Möglichkeit zur Fütterung von Schweinen ohne Supplementierung von mineralischen Phosphaten

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Pigs don´t produce measurable amounts of endogenous phytase, and therewith they are not able to release sufficient phytate-bound phosphorous (P) from plant-based feed ingredients, to satisfy their nutritional requirements. Supplementation with a hybrid 6-phytase has been shown to increase P and calcium availability in pig diets with reduced contents of supplemented P (sP) [1]. The hypothesis of the present study was, that with the use of microbial phytase, supplementation of pig diets with P could not be required anymore provided that a sufficient amount of phytate-bound P substrate is in the diet. Thus a trial was conducted to confirm the viability of producing pigs from weaning to slaughter using phytase supplemented diets without sP.

Methods: 48 newly weaned piglets ([Large white x Landrace] x Pietrain; 25 days of age; 7.4 kg BW; ½ entire males and ½ females) were randomly distributed by initial body weight into 24 pens (2 piglets of the same sex per pen) during the postweaning phase (0-43 d), and moved into 48 individual pens during the fattening phase (44-144 d). The trial was arranged according to a randomised block design with 6 blocks of initial body weight for each sex, and 2 treatments. Between days 0-7, all pigs were offered a common pre-starter 1 diet without sP but with 2000 FTU phytase/kg. Between days 8-144 pigs were offered the two experimental treatments in all feeding phases: control (C) diets which included sP as monocalcium phosphate (MCP) and test diets (PHY) without sP but supplemented with phytase. The five experimental feeding phases were: pre-starter-2 (8-21 d), starter (22-43 d), grower-1 (44-74 d), grower-2 (75-112 d), and finisher (113-144 d), which were formulated to provide 2.5, 2.7, 2.8, 3.0, and 3.0 g/kg feed of phytate-P, respectively. The C-diets contained 7.0, 7.3, 6.7, 6.5, 5.9 g/kg feed of Ca and 6.9, 6.5, 6.1, 5.9, 5.8 g/kg feed of tP whereas Phy-diets contained 4.9, 5.2, 4.8, 5.1, 4.9 g/kg feed of Ca and 5.1, 4.7, 4.4, 4.7, 5.0 g/kg feed of tP, respectively. All diets contained wheat, barley, soybean meal, rapeseed meal and sunflower meal as main ingredients. For the PHY treatment, phytase was added at 2000, 2000, 1000, 500 and 300 FTU/kg in pre-starter-2 and starter (postweaning phase), grower-1, grower-2 and finisher (fattening phase), respectively. Performance was evaluated during the postweaning, fattening and overall periods, and at the end of the trial, the left Os metacarpale III bones were obtained and analysed for dry weight and ash content. Treatment means were compared by ANOVA using the GLM procedure of SAS, considering the effects of block, sex and treatment and using the initial pen as the experimental unit (n=12).

Results: No statistically significant differences between the C and PHY treatments were observed during the postweaning phase for average daily weight gain (488 vs 485 g/d; P = 0.91), average daily feed intake (659 vs 680 g/d; P = 0.46), or feed to gain ratio (1.35 vs 1.39; P = 0.15). Similarly, average daily weight gain (897 vs 859 g/d; P = 0.26), average daily feed intake (2198 vs 2098 g/d; P = 0.16), or feed to gain ratio (2.46 vs 2.46; P = 0.88) did not differ during the fattening phase either. Over the whole trial, no effects of treatment were observed on average daily weight gain (775 vs 752 g/d; P = 0.28), average daily feed intake (1734 vs 1718 g/d; P = 0.79) and feed to gain ratio (2.24 vs 2.29; P = 0.25). At the end of the trial, the dry weight of the Os metacarpale III bone (17.6 vs 17.8 g; P = 0.68) and its ash content (42.6 vs 41.6 %; P = 0.79) did not differ between treatments.

Conclusions: It is concluded that microbial hybrid 6-phytase can completely replace sP sources in diets for pigs from weaning to slaughter without negative impacts on performance or bone mineralisation. Feeding without the non-renewable sources of iP will in combination with phytase supplementation minimize the P excretion and reduce the environmental impact.

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39.

Comparative aspects of protein and energy metabolism in early lactation Holstein and Simmental cows under the same feeding conditions

Vergleichende Betrachtung des Protein- und Energiestoffwechsels von Holstein und Fleckvieh Kühen in der Frühlaktation unter gleichen Fütterungsbedingungen

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The dual-purpose breed German Simmental (SI) is generally considered to be less prone to metabolic disease than the single purpose dairy breed German Holstein (HF). However, comparative studies often are biased by different production conditions these two breeds are usually faced with.

Methods: The present study realized a comparative approach of SI (n = 9) and HF (n = 15) kept under identical feeding and management conditions, and with similar milk yield [kg ECM 100 (mean ± SEM): HF: 4025.5 kg ± 146.7; SI: 3646.4 ± 176.3; P = 0.114]. Cows were fed a partial mixed ration (PMR) based on grass and corn silage ad libitum; both together containing 5.6 MJ NEL/kg DM for dry cows and 6.7 MJ NEL/kg DM for lactating cows. Transition feeding, starting two weeks prior to calving with increasing amounts of concentrate (8.1 MJ NEL/kg DM) ended up with 2 kg per day. Lactating cows additionally received transponder-controlled individual concentrate (8.7 MJ NEL/kg DM) adjusted to their individual milk yield. Within the first 30 days of lactation, 150 ml of propylene glycol and glycerol (ProGlyc®, VILOFOSS, Neuenkirchen-Vörden, Germany) per day were added to the ration. All cows had ad libitum access to water. Animals were clinically examined and sampled at day -42 ante partum (ap) and day 21 post partum (pp). Body weight (BW) was assessed from day 8 to day 100 pp. Classical clinical chemical parameters were analyzed in plasma (glucose, BHBA: Cobas c 311, Roche Diagnostics, Mannheim, Germany; insulin: ELISA Mercodia, Uppsala, Sweden), and proxies of insulin sensitivity were calculated. A targeted metabolomics approach (AbsoluteIDQ p180, Biocrates Life Science AG, Innsbruck, Austria) was applied. Data were evaluated by univariate data analysis, and metabolomics data were also subjected to multivariate data analysis using the MetaboAnalyst 3.5 software. $P < 0.05$ was considered significant.

Results: All cows enrolled were clinically healthy at sampling. There was a significant interaction of breed and time for BW after calving. Whereas HF cows lost body weight between day 8 and 60 pp ($P = 0.009$), BW in SI was maintained ($P = 1.0$). Individual concentrate allocation and FCR until day 100 pp did not differ ($P > 0.2$) At day 21 pp, concentrations of BHBA in HF were higher ($P = 0.049$), and RQUICKI-BHB was lower ($P = 0.048$) than in SI, whereas concentrations of insulin and glucose did not differ. Concentrations of branched chain amino acids (BCAA), were also higher in HF compared to SI at day 21 pp ($P = 0.042$). Further creatinine was influenced by breed ($P < 0.001$) leading to higher plasma concentrations at day 42 ap and day 21 pp in SI.

Conclusions: High concentrations of BCAA and low concentrations of creatinine might point to a higher protein turnover in HF than in SI adapting to lactation. Furthermore, BCAA are associated with insulin resistance in other species and therefore possibly indicate more effective insulin action in SI compared to HF. This is supported by lower RQUICKI-BHB in HF at day 21 pp [1].

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40.

Bile acids concentrations and their receptor expression in subcutaneous adipose tissue of dairy cows with high versus normal body condition

Gallensäurekonzentrationen und deren Rezeptorexpression im subkutanem Fettgewebe von Milchkühen mit hoher oder normaler Körperkondition

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Cows with high body condition before calving mobilize more body reserves after parturition than thinner cows. Besides their role in lipid digestion, bile acids (BA), both the primary ones as well as the secondary BA generated by the intestinal microbiome, reaching the circulation and exert also endocrine effects via BA receptors, intervening in energy homeostasis [1]. Recently we observed 7 BA in subcutaneous adipose tissue (scAT), i.e. cholic acid (CA), glycocholic acid (GCA), taurocholic acid (TCA), glycochenodeoxycholic acid (GCDCA), taurochenodeoxycholic acid (TCDCA), glycodeoxycholic acid (GDCA), and taurodeoxycholic acid (TDCA) [2]. Based on their function in controlling glucose and lipid metabolism, we tested the hypothesis that BA concentration and BA receptor-mRNA abundance differ in scAT from dairy cows with either high or normal body condition around parturition.

Methods: Fifteen weeks (wk) ante partum (ap), German Holstein cows were classified by their body condition score (BCS) and backfat thickness (BFT) into either a high (HBCS; n = 19) or a normal BCS (NBCS; n = 19) group. Cows were fed differently from 15 wk ap to 7 wk ap (HBCS: 7.2 NE_L MJ/kg dry matter (DM)); NBCS: 6.8 NE_L MJ/kg DM) to reach the targeted differences in BCS and BFT (NBCS: BCS < 3.5, BFT < 1.2 cm; HBCS: BCS > 3.75, BFT > 1.4 cm) until dry-off at 7 wk ap. During the dry period and the subsequent lactation, both groups were fed identical diets (5.4-5.8 and 7.1 MJ NE_L/kg DM, respectively). Biopsies from scAT were collected at wk -7, 1, 3, and 12 relative to parturition and were assayed by LC-ESI-MS/MS with the BiocratesTM BA Kit (BIOCRATES Life Sciences AG, Austria). Using the Biomark HD 96.96 system (Fluidigm Co., San Francisco, CA, USA), the mRNA abundance of BA receptors was measured in scAT. Data (mean ± SEM) were analyzed by a linear mixed model with repeated measurements including Bonferroni correction for multiple comparison (SPSS 28). Group, time, and their interaction were classified as fixed effects, whereas the individual cow was considered as a random factor.

Results: In scAT, concentrations of CA, GDCA, and GCA were lower ($P < 0.001$) ap and at wk 1 post partum (pp) compared to wk 3 and 12 pp irrespective of grouping. Moreover, GCDCA concentrations were lower ap compared to pp ($P < 0.001$). The mRNA abundance of the membrane-bound receptors Takeda G protein-coupled receptor 5 (TGR5), Sphingosine-1-Phosphate Receptor 2 (S1PR2), and Cholinergic Receptor Muscarinic 2 (CHRM2) as well as the nuclear receptor Vitamin-D Receptor (VDR) and Retinoid X Receptor α (RXRα) were detectable in scAT. Farnesoid X Receptor (FXR) and Constitutive Androstane Receptor (CAR) mRNA were detectable only in few AT samples ($n \leq 4$). Irrespective of the group, the mRNA abundance of TGR5 and CHRM2 increased from ap to 3 wks pp ($P < 0.001$). The mRNA abundance of RXRα was highest at wk 12 pp ($P < 0.001$). Group differences were observed for the mRNA abundance of S1PR2 with 2.12-fold higher values in NBCS than in HBCS cows ($P = 0.039$).

Conclusions: The observed changes in both, BA concentrations and their receptor mRNA abundance in scAT, with time and partly with body condition, point to a gut-adipose-tissue axis and a potential role of BA in lipid metabolism. In mature 3T3-L1 adipocytes, BA acting via specific receptors were suggested to inhibit lipolysis [3]. However, in the present study, lithocholic acid (LCA), serving as the main BA ligand for VDR, was not detectable in scAT and several BA receptors may bind other ligands besides BA. Therefore, the specific role of BA and their receptors in lipid metabolism during the periparturient period of dairy cows needs to be further elucidated.

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41.

Usability of semi-volatile organic compounds from different biological matrices for identification of diet specific metabolic profiles in dairy cows

Verwendbarkeit von halbflüchtigen organischen Verbindungen aus verschiedenen biologischen Matrizen zur Identifizierung von ernährungsspezifischen Stoffwechselprofilen bei Milchkühen

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Metabolomics approaches can provide snapshots of the metabolism after the ingestion of different diets [1]. Semivolatile organic compounds (SVOC) have a boiling point of 240°C - 380°C, like aldehydes, ketones and amines. They represent a subcategory of the metabolome and can be detected in the gas phase of biological matrices [2]. The SVOC are not captured by current metabolomics approaches, but they provide a high potential to find new potential biomarkers [1]. Therefore, the goal of the present study was to examine the usability of SVOC to expand metabolomics approaches. To accomplish this, various biological matrices from cows fed a hay or silage-based diet were analysed for SVOC at different time points using an untargeted metabolomics approach. Correlations in the metabolite profiles of the different matrices were investigated.

Methods: Sixteen lactating Holstein Friesian and Red Holstein cows (milk yield: 31.0 ± 4.8 kg/d; 79 ± 31 d in milk) were blocked by milk yield, days in milk and lactation number, and were assigned to two diets. They were either fed hay (n =8) or a silage-based diet (n = 8) composed of maize silage, grass silage and hay (44:43:13%). All cows were supplemented with a protein concentrate and an energy concentrate to meet the estimated nutritional requirements. They received 300 g/d of a mineral–vitamin mixture. The study lasted 16 weeks, divided into four periods of four weeks each. Samples were taken in the last week of each period: milk as aliquot from one morning and one evening milking, blood from the vena jugularis, urine from stimulated micturition and ruminal fluid using a stomach tube. Samples were derivatised and analysed by an 8890/7250 GC-MS qTOF system (Agilent Technologies, Santa Clara, USA). Multivariate analyses to discriminate between diets (partial least squares-discriminant analysis (PLS-DA, sparse PLS-DA) and correlation analyses ($r > 0.7$) were performed for each of the four periods separately (R-packages MixOmics and ropls; MetaboAnalyst). For PLS-DA, the predictive ability parameter ($Q2 > 0.5$ was considered as sufficiently predictive) and the goodness of fit (R2Y) were used to describe the model quality.

Results: TheSVOC metabolic profiles differed between hay and silage fed cows. For ruminal fluid, the discrimination was very good over all four periods (Q2/R2Y values): 0.68/0.99, 0.73/0.99, 0.59/ 0.99, 0.71/0.99). A discrimination based on serum (Q2Y/R2Y values: -0.1/0.8, 0.65/0.99, 0.73/0.99, 0.67/0.99) and urine (Q2/R2Y values: 0.35/0.99, 0.52/0.99, 0.59/0.99, 0.67/0.99) was possible from the second period on. In milk, the discrimination (Q2/R2Y values: 0.67/0.99, 0.43/0.99, 0.03/0.77, 0.59/0.99) was less consistent across periods. Discriminating metabolites were hydroxyphenylpropanoic acid and hydroxyphenylacetic acid in ruminal fluid, tryptophan and arginine in serum, D-galactose and myo-inositol in urine, rhamnopyranose and galactosamine in milk. Most discriminating metabolites of each biological matrix correlated ($r > 0.7$) among each other and were higher in hay fed cows compared to silage fed cows.

Conclusions: Cows fed hay or silage differed in their SVOC metabolic profiles, which indicates the general usability of SVOC for diet-specific metabolomics approaches. The SVOC from ruminal fluid showed the best suitability, followed by serum, urine and milk. The order reflects the nutrient flow and the metabolisation, and suggests different response times of the biological matrices to the diets. Metabolites in milk might be more influenced by external factors (e.g. stress, environment). The strong correlations demonstrated the links of metabolites among the different biological matrices. In future research, we want to use SVOC profiles and their pathways to describe the metabolic status of cows, e.g. to detect metabolic disorders, as is already known for acetone to detect ketosis.

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42.

Investigations of the effect of dietary insect larvae meal from the black soldier fly on the caecal microbiome, the liver transcriptome and the plasma metabolome of broilers

Untersuchungen zur Wirkung von diätetischem Insektenlarvenmehl der schwarzen Soldatenfliege auf das Zäkummikrobiom, das Lebertranskriptom und das Plasmametabolom von Broilern

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Alternative protein sources are increasingly needed for animal production to face the challenge of worldwide growing demand for products of animal origin, and the increasing shortage of natural resources, such as arable land and water, required for the production of dietary protein sources. In this regard, protein-rich insect biomass obtained from industrialized mass-rearing of appropriate edible insects, such as *Hermetia illucens* (HI), has been recognized as a reasonable strategy to provide broiler and pigs with dietary protein. One important prerequisite for the use of IM as feed for farm animals is that it does not cause any adverse effects on animal´s performance and metabolic health. While a large number of studies has reported that the use of HI larvae meal as the main protein source does not impair performance of broilers, comprehensive investigations on the metabolic effects of HI larvae meal in broilers are lacking. Against this background, the present study aimed to comprehensively describe the effects of HI larvae meal on the liver transcriptome, the plasma metabolome and the caecal microbiome of broilers.

Methods: 100 male, 1-day-old Cobb 500 broilers were randomly assigned to three groups and fed three different nutrient adequate diets, which contained either 0% (HI-0), 7.5% (HI-7.5) or 15% (HI-15) HI larvae meal in a three-phase feeding system for 35 days. After killing the animals, blood plasma, liver and ileal and caecal digesta were collected and stored at -80°C pending analysis. The liver transcriptome was analyzed using a chicken whole-genome gene array. The caecal digesta microbial community was analyzed by 16S rRNA-based high-throughput sequencing. Caecal digesta concentrations of short-chain fatty acids (SCFA) were analyzed by gas-chromatography flame-ionisation detection (GC-FID). Targeted metabolomics of plasma was carried out by mass spectrometry using the MxP Quant 500 kit. Normally and not-normally distributed data were analyzed by one-way ANOVA and Kruskal-Wallis test, respectively.

Results: Body weight gain, final body weight, feed intake, and feed:gain ratio were not different between groups, whereas absolute and relative breast muscle weights were higher in group HI-15 than in group HI-0 ($P<0.05$). Caecal microbial α-diversity was higher in group HI-15 than in group HI-0 ($P<0.05$), but did not differ between group HI-7-5 and group HI-0. Taxonomic analysis revealed differences among groups in the abundance of diverse low-abundance bacterial genera, such as Shuttleworthia (decreased in group HI-15 compared to group HI-0, $P<0.05$) and Caproicipproducens (increased in group HI-15 compared to group HI-0, $P<0.05$). Concentrations of total SCFA, acetic acid, propionic acid and isovaleric acid in caecal digesta did not differ among groups, whereas those of butyric acid, isobutyric acid and valeric acid were lower in group HI-15 than in group HI-0 ($P<0.05$). Considering the two filter criteria (fold change >1.3 or <-1.3; $P<0.05$), liver transcriptomics revealed a total of 65 transcripts to be differentially expressed (32 upregulated, 33 downregulated) between group HI-15.0 vs. HI-0 ($P<0.05$). Out of these genes, only seven and three genes were found to be upregulated and downregulated, respectively, greater 1.5-fold indicating a moderate effect on the liver transcriptome. The concentrations of 176 plasma metabolites, most of which were lipid species, were found to differ among groups ($P<0.05$) using targeted metabolomics. Particularly, C14:0-containing triglyceride species were increased in groups HI-15.0 and HI-7.5.

Conclusions: Dietary inclusion of HI larvae meal as a dietary protein source in broiler diets enhances breast muscle weights, causes a favorable increase of caecal microbial diversity, markedly alters the plasma metabolome, but induces only moderate changes in the liver transcriptome.

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43.

Effect of replacement of soybean oil by *Hermetia illucens* fat in broiler diets on the gut microbiome, liver transcriptome and liver and plasma lipidomes of broilers

Wirkung des Austauschs von Sojaöl durch Hermetia illucens-Fett in Broilerdiäten auf das Darmmikrobiom, das Lebertranskriptom und das Leber- und Plasmalipidom von Broilern

Schäfer L., *Ringseis R., Grundmann S. M., Maheshwari G., Höring M., Liebisch G., Most E., Eder K. – Giessen / Bonn / Regensburg

Processed insect biomass has been recently approved as a feed for poultry and pigs with the aim of improving sustainability of food systems. Owing to its high protein content, research dealing with the feed potential of insect biomass has primarily focused on its role as a source of protein. In contrast, the feed potential of insect fat is generally less explored and knowledge about the suitability of insect fat as a fat source specifically in broiler diets is still limited. In particular, in-depth analysis of the effects of insect fat on the gut microbiome and intermediary metabolism is lacking. Against this background, the present study aimed to comprehensively investigate the effect of partial (50%) and complete replacement of soybean oil with HI larvae fat in broiler diets on the gut microbiome, liver transcriptome and liver and plasma lipidomes.

Methods: 100 male, 1-day-old Cobb 500 broilers were randomly assigned to three groups and fed three different nutrient adequate diets, which varied only in the fat source (group HI-0: 0% HI larvae fat and 5% soybean oil; group HI-2.5: 2.5% HI larvae fat and 2.5% soybean oil; group HI-5.0: 5.0% HI fat and 0% soybean oil), in a three-phase feeding system for 35 days. After killing the animals, blood plasma, liver and digesta from ileum and cecum were collected and stored at -80°C pending analysis. The liver transcriptome was analyzed using an Affymetrix GeneChip Array (Chicken Gene 1.0 ST). The caecal digesta microbial community was analyzed by 16S rRNA-based high-throughput sequencing. caecal digesta concentrations of short-chain fatty acids (SCFA) were analyzed by gas-chromatography flame-ionisation detection (GC-FID). The liver and plasma lipidomes were analyzed by direct flow injection-mass spectrometry. Analysis of fatty acid composition of liver total lipids was carried out by GC-FID. Normally and not-normally distributed data were analyzed by one-way ANOVA and Kruskal-Wallis test, respectively.

Results: Body weight gain, feed intake, and feed:gain ratio during the whole period and apparent ileal digestibility of crude fat and gross energy were not different between groups. Caecal microbial diversity did not differ between groups and taxonomic analysis revealed differences in the abundance of only four low-abundance bacterial taxa among groups; the abundances of Actinobacteriota, Coriobacteriia, Coriobacteriales and Eggerthellaceaein caecal digestawere lower in group HI-5.0 compared to group HI-2.5 ($P<0.05$). Concentrations of total and individual SCFA in the caecal digesta were not different between the three groups. Liver transcriptomics revealed a total of 55 and 25 transcripts to be differentially expressed between groups HI-5.0 vs. HI-0 and groups HI-2.5 vs. HI-0, respectively ($P<0.05$). The concentrations of most lipid classes and the sum of all lipid classes in liver and plasma were not different between groups. Pronounced differences between groups were seen with regard to the composition of individual lipid species within the different lipid classes in both, liver and plasma; e.g., relative proportions of triglyceride species with zero and one double bonds were higher in group HI-5.0 than in group HI-0, while those with five and six double bonds were lower in group HI-5.0 than in group HI-0 ($P<0.05$). Within hepatic total lipids, the proportions of C12:0, C14:0 and C16:1 were higher in group HI-5.0 than in groups HI-2.5 and HI-0, whereas the proportion of C18:3 n-3 was lower in groups HI-5.0 and HI-2.5 than in group HI-0 ($P<0.05$).

Conclusions: Comprehensive analysis of the effects of dietary replacement of soybean oil with HI larvae fat revealed only very modest, but not any adverse effects in broilers. The findings of this study suggest that HI larvae fat can be used as an alternative fat source in broiler diets, which makes broiler production more sustainable through the exclusion of soybean oil and the utilization of HI fat from regional production.

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44.

Effect of replacement of soybean oil by *Hermetia illucens* fat in broiler diets on the breast muscle lipidome and the concentrations of cholesterol and phytosterol oxidation products in heat-processed breast muscle

Wirkung des Austauschs von Sojaöl durch Hermetia illucens-Fett in Broilerdiäten auf das Brustmuskellipidom und die Konzentrationen an Cholesterol- und Phytosteroloxidationsprodukten in hitzebehandeltem Brustmuskel

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Replacement of soybean oil by insect fat from *Hermetia illucens* (HI) has been reported to increase the proportions of saturated fatty acids (SFA) and decrease those of polyunsaturated fatty acids (PUFA) in total lipids of breast and thigh meat in broilers [1]. Since PUFA are highly susceptible to oxidation, meat obtained from broilers fed HI fat as dietary fat is expected to be more stable against lipid peroxidation during cooking or frying than meat from broilers fed soybean oil as dietary fat. Unlike thiobarbituric acid-reactive substances (TBARS), which are widely used parameters of lipid peroxidation, oxidation products of cholesterol (COP) and phytosterols (POP) are much more specific indicators of lipid oxidation [2]. In addition, COP and POP are relevant with respect to human health because they are implicated in the development of coronary heart disease or cancer. Considering this, the present study aimed to test the hypothesis that replacement of soybean oil by HI larvae fat in broiler diets reduces the formation of COP and POP in heat-processed breast muscle of broilers.

Methods: 100 male, 1-day-old Cobb 500 broilers were randomly assigned to three groups and fed three different nutrient adequate diets, which varied only in the fat source (group HI-0: 0% HI larvae fat and 5% soybean oil; group HI-2.5: 2.5% HI larvae fat and 2.5% soybean oil; group HI-5.0: 5.0% HI fat and 0% soybean oil), in a three-phase feeding system for 35 days. After killing the animals, the breast muscle was excised and weighted. Several aliquots were snap-frozen in liquid nitrogen and stored at -80°C pending analysis. For heat-processing, a muscle-aliquot was thawed overnight, and the thawed muscles were heated in a drying oven at 170°C for 50 min. After cooling the muscles to ambient temperature, several aliquots were collected for analysis. Analysis of fatty acid composition and lipidomic analysis of major lipid classes in unheated breast muscle were carried out by gas-chromatography (GC) flame-ionisation detection and direct flow injection-mass spectrometry, respectively. Concentrations of COP/POP and TBARS were determined by GC-mass spectrometry and fluorescence spectrometry, respectively. Normally and not-normally distributed data were analyzed by one-way ANOVA and Kruskal-Wallis test, respectively.

Results: Body weight gain, eviscerated carcass weight and dressing percentage of the broilers were not different between groups. Breast muscle weight was higher in group HI-5.0 than in group HI-0 ($P<0.05$). The proportions of total SFA were higher and those of total PUFA were lower in unheated breast muscle total lipids of group HI-5.0 than in groups HI-2.5 and HI-0 ($P<0.05$). In unheated breast muscle, the concentration of triacylglycerols was 46% and 53% lower in groups HI-2.5 and HI-5.0, respectively, than in group HI-0 ($P<0.05$), whereas all other lipid classes detected did not differ among groups. Within triacylglycerols, relative proportions of lipid species with zero, one and two double bonds were higher in group HI-5.0 than in group HI-2.5 and in group HI-0, while those with three and more double bonds were lower in group HI-5.0 than in group HI-2.5 and in group HI-0 ($P<0.05$). Lipid-adjusted concentrations of TBARS, 7α-OH cholesterol, 7β-OH cholesterol, 7-ketocholesterol, total COP and 7α-OH campesterol in heat-processed breast muscle were lower in group HI-5.0 than in group HI-0 ($P<0.05$), but did not differ between groups HI-2.5 and HI-0.

Conclusions: Complete replacement of soybean oil with HI larvae fat in broiler diets strongly alters the fatty acid composition of breast muscle total lipids and reduces lipid peroxidation of the breast muscle during heat-processing. This suggests that the shelf life of meat produced from broilers fed HI larvae fat instead of soybean oil is improved and consumers ingesting such heat-processed broiler meat are less confronted with detrimental COP.

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45.

Effect of different forage proportions in diets of fattening bulls on protein, fat, energy, phosphorus and potassium accretion

Einfluss von unterschiedlichen Grundfutteranteilen in der Ration von Mastbullen auf den Protein-, Fett-, Energie-, Phosphor- und Kaliumansatz

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The current recommendations for energy and nutrient supply for growing cattle are under evaluation. Therefore, the aim of this study was to investigate the nutrient, energy and phosphorus/potassium accretion of fattening bulls with varying forage proportions in the diet in order to generate data for modern male Holstein genetics.

Methods: Fifty-six fattening bulls of the German Holstein breed were slaughtered at different body weights (mean 494 kg, minimum 218 kg; maximum 787 kg). Eight animals were slaughtered at the start of the experiment and served as an initial group (IG). The remainder animals were divided into two feeding groups. Over the experimental period (forty-three weeks in total) the animals were slaughtered after different weeks distributed over the entire experimental period. One half of the animals received a ration with a high forage proportion (HF, 80% forage on dry matter (DM) basis) and the remainder animals a low forage proportion (LF, 40% forage on DM basis) in the ration. The ration was fed as a total mixed ration and contained corn silage as the forage component and concentrate in both feeding groups. The individual dry matter intake was recorded daily and the animals were weighed once a week. During the slaughter process bull‘s body parts were assigned to different fractions, which were homogenized, weighted and analyzed for DM, fat, protein and ash content for determination of body composition (BC). The empty body weight (EBW) was calculated as the whole body of the bull without claws off cut, ingesta and content of urinary and gall bladder. BC data were analysed with linear or non-linear regression models to obtain the protein, fat, energy, phosphorus and potassium accretion by using the Statistica software version 13. The BC data of the IG were used as starting values for the HF and LF group. In order to describe the accuracy of the estimation the residual standard deviation (RSD) was calculated.

Results: are related to a weight range from 200 to 800 kg live weight. Protein accretion was 116 g per kg live weight gain for the HF group and 119 g per kg for the LF group. Converted to nitrogen (N) the animals of the HF group retained 18.6 g N and the animals of the LF group retained 19.1 g N per kg of live weight gain. Fat accretion was 291 g per kg live weight for the HF group and 316 g per kg live weight gain for the LF group. Energy accretion was 13.8 MJ per kg live weight for the HF group and 14.9 MJ per kg live weight gain for the LF group. Phosphorus and potassium accretion were almost equal between the two groups. The animals of the HF group accreted 7.5 g phosphorus and 1.1 g potassium and the animals of the LF group 7.8 g phosphorus and 1.2 g potassium per kg live weight gain.

Conclusions: Under conditions of the present experiment the different forage proportions in the ration influenced the accretion of protein, fat, energy, phosphorus and potassium of fattening bulls of the German Holstein breed only marginal.

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46.

Growth performance and economic sustainability of Simmental fattening bulls fed dry or corn silage-based total mixed rations

Wachstumsleistung und wirtschaftliche Nachhaltigkeit von Fleckvieh-Mastbullen, die mit Trocken- oder Maissilage gefüttert werden

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Bull fattening diets in Europe and most developed countries worldwide are traditionally based on corn silage, starch-rich and high-energy supplemental feed. International and regional crop and corn silage yields are likely to be severely impacted by global climate change, such as changing precipitation patterns and insufficient water availability. Considering the effects of climate change on crop yields, feed availabilities, and price volatility, especially in bull fattening, new and adapted feeding strategies need to be considered. Therefore, the objective of this study was to compare feed intake, growth performance, and economic sustainability of Simmental bulls fed either a conventional corn silage-based ration (CONVL) or a dry (DRY) total mixed ration (TMR) until they reached a final body weight of approximately 750 kg.

Methods: For ten months, 24 bulls (215 ± 10 kg BW) were randomly assigned to one of two TMR feeding groups (n = 12 per group). The DRY-TMR was primarily characterized by the dietary fiber source, which was based solely on straw and by-products. The diets were formulated and balanced based on the Cornell Net Carbohydrate and Protein System [1]. The main differentiator between the DRY and CONVL diets was the absence or presence of corn silage. As a result, the dry matter content (DM) of the CONVL TMR was 44.0% and lower than the DM content of the DRY-TMR, which was 72.5%. For the evaluation of the particle size of the feed, duplicate samples were collected from each group of feed bunks eight weeks after the start of each feeding. A repeated-measures model was fitted to the data using PROC MIXED from SAS (version 9.4). The model consisted of treatment, time, and the interaction of treatment and time as fixed effects and bulls and pen as random effects. Veterinarians examined the health status of the bulls weekly, and no clinical abnormalities or unexpected losses were noted. After 272 days of fattening, bulls were slaughtered.

Results: Feed intake, average daily gain (ADG)/DM intake ratio, and nutrient intake were affected by treatment, time, and their interaction ($P < 0.01$). Compared with CONVL bulls, animals fed the DRY-TMR consumed more non-fibre carbohydrates and rumen undegradable neutral detergent fibre, showing lesser dry and fresh matter intake and less ME and physically effective neutral detergent fiber (peNDF) intake. The fattening period could be shortened by 60 days due to the high ADG in both treatment groups (DRY-TMR = 1.87 kg/day and CONVL = 1.84 kg/day). Both treatments achieved a positive profit margin (598 ± 28 €/bull). While total income per bull and dressing percentage did not differ between treatments, the substantially higher feed costs ($P < 0.005$) of the DRY-TMR resulted in a higher ($P = 0.04$) income over feed cost in favour of the CONVL treatment group. Despite the higher dietary costs, the reduction in fattening duration and the improved ADG/DMI ratio ($P < 0.01$) of the DRY-TMR contributed to reduced absolute feed quantity requirements while allowing to profit from the genetic growth potential of the bulls.

Conclusions: Based on the positive profit margin, DRY-TMR solutions for fattening bulls based on straw and by-products can be seen as a promising alternative feeding strategy and may contribute to both the economic and ecological sustainability of fattening farms.

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47.

Effects of particle size reduction of meadow hay on feed intake, apparent total tract digestibility of nutrients and performance of organic dairy cows

Effekte von gehäckseltem Wiesenheu auf die Futteraufnahme, die scheinbare Verdaulichkeit der Nährstoffe sowie die Leistung von Milchkühen unter ökologischen Bedingungen

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The steadily increasing population and the resulting demand for food encourages the animal industry to reduce the use of human-edible feed ingredients. Ruminants' ability to utilize forages allows for the replacement of concentrates, with benefits for the animals' health but also for the net human food supply (Wilkinson, 2011). In order to meet the animals' nutrient requirements, a high forage intake is necessary. This can, however, be limited due to the voluminous nature of forages. A recent study by Haselmann et al. (2019) found that reduced particle size included in a TMR stimulated feed intake and improved apparent total tract digestibility (ATTD). Yet, it is not clear whether the same strategy would work when forages are fed separately, as typically practiced on small-scale organic farms in Austria. We hypothesized that the particle size reduction of hay results in an increase of dry matter intake (DMI), ATTD and milk performance.

Methods: 18 lactating Holstein cows were balanced by body weight (675 kg), days in milk (125d), number of lactations (3.6) and average milk yield (23 kg) and allocated to two feeding groups. Both groups were fed meadow hay from the 2nd cut, consisting of 138 g CP, 497 g NDF, and 5.8 MJ NEL per kg DM, either of conventional length (CON), or with a reduced particle size (RED). To obtain the RED, the meadow hay was chopped to a theoretical length of 0.5 cm and particle size distribution was measured with a Penn State Particle Separator (PSPS). Individual feed intake was recorded using CALAN gates. After four weeks of adaptation to the feeders and a covariate feeding period (7d) with a TMR, both groups were switched to hay treatments for 34d, whereby the first 14d served as an adaptation period. Cows were offered fresh hay after milking (06:00 and 17:00) allowing for 10% feed refusals. Additionally, every cow received 3.6 kg DM/d concentrate, offered in equal shares before milking. Milk yield was recorded automatically, feed intake was determined once a day, and ATTD was defined using acid soluble ash as an external marker. Nine faecal samples per cow were collected from the rectum at an 8h interval, pooled, and freeze dried for chemical analysis. Data was analyzed in an ANOVA with a proc mixed model using SAS 9.4., considering the effect of the feeding group, day and covariates for the corresponding parameter, while cow nested within group was considered as a random effect.

Results: Hay particles retained on the screens 19, 8, and 4 mm as well as the pan of the PSPS in the RED group accounted for 19, 22, 23, and 37% fresh matter basis, respectively. Cows in the CON group reached DMI levels of (20.8 kg/d) while the level increased slightly (+1.05 kg/d) when fed the RED diet ($P = 0.28$), resulting in an increase (+0.6 kg/d) of milk yield in the RED group ($P = 0.59$). The ATTD of nutrients decreased significantly when feeding RED hay, but faecal DM content remained constant. The digestibility of DM and OM decreased by 4.4 and 4.7% points, respectively. The reduced particle size of the hay, however, improved the cows' energy balance numerically (103.7 vs 107.1%, $P = 0.52$), resulting in a modest increase ($P = 0.46$) of energy-corrected milk (ECM) production (+0.8 kg/d). Feed conversion efficiency (kg ECM/kg DMI) and N use efficiency did not differ between the control and the experimental group.

Conclusions: The results indicate that there was a numerical increase in DMI and milk yield when feeding reduced particle sizes of hay separately. The decrease in digestibility in the RED cows suggests an increased passage rate, potentially due to the high fraction of particles <4 mm due to chopping. While reduced bulkiness has the potential to increase the DMI and therefore performance of organic dairy cows, more research with different increments of particle size reduction is necessary to optimize that potential.

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48.

Effect of grazing management on nitrogen use efficiency of lactating dairy cows grazing temperate semi-natural grasslands

Einfluss des Weidemanagements auf die Stickstoffnutzungseffizienz laktierender Milchkühe auf temperaten, semi-natürlichen Weiden

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In low-input dairy farms in temperate grassland-dominated regions, pasture herbage and freshly harvested forage are main feed sources during the grazing season. A challenge of these forage-rich diets is the lower efficiency with which ingested nitrogen (N) is converted to milk crude protein (CP), mainly due to high concentrations of rapidly degradable CP and the, at least partly, low energetic value of fresh forages. The aim of the present study was to identify grazing management factors influencing the N use efficiency of cows in dairy farms using semi-natural grasslands for grazing.

Methods: Nine commercial organic dairy farms in Southwest Germany were visited for one or two periods per year (2019, 2020) á 11 days. Pasture biomass was measured and its botanical composition assessed once per period. Supplement feedstuffs offered in barn were weighed and sampled daily. Dry matter (DM) intake was estimated via a double marker technique using titanium dioxide as external marker [1] in 8 to 30 lactating cows per farm and period (n = 323 cows in total). Milk, faeces, and urine samples were taken daily from each cow during Days 6-11 and analysed for N. Additionally, milk was analysed for urea, urine for creatinine (C) and purine derivatives (PD), feed for DM, organic matter, metabolisable energy (ME), and neutral-detergent fibre (NDF), and faeces for DM, organic matter, and titanium dioxide. Pasture DM intake was estimated by subtracting DM intake of supplemental feed from total DM intake of each cow. Using the R package 'bootStepAIC' [2], backward elimination was perfomed on 1000 bootstrap resamples to identify significant grazing management factors among 17 pre-selected variables [3]. This analysis was done for seven N use efficiency indicators as outcome variables: milk N use efficiency (milk N production in % of N intake), faecal and urinary N excretions (% of N intake), milk urea-N (mg/dL), and urinary N:C, PD:C, and PD:N ratios. Consequently, model inclusion frequency for each management factor, and frequency of positive and negative signs of resulting regression coefficients were calculated.

Results: Mean (± standard deviation) total and pasture DM intakes, as well as milk yield were 21.0 (± 3.2), 11.3 (± 4.8), and 23.9 (± 5.4) kg/d. The mean CP concentration of pasture herbage was 16.7 ± 2.93 g/100 g DM. The ingested N was mainly excreted as urine (48.9 ± 9.77%), and to a smaller extend as faeces (26.4 ± 4.96%), while milk N use efficiency averaged 24.7 (± 5.91) % across all farms, years, and periods. Daily DM intake of supplement feeds, share of fresh forage and hay of total supplement DM intake, and ME and NDF concentrations of pasture herbage were most frequently selected to explain multiple N use efficiency indicators. An increase of these selected factors improved N use efficiency, except for DM intake from supplement feed, which was negatively related to N use efficiency ($p \leq 0.01$ for all factors). For several N use efficiency indicators, herb-rich swards and full-time grazing increased N use efficiency, as compared to grass-rich (> 60% grasses in aboveground fresh matter) or balanced swards (≤ 60 and > 45 % grasses) or grazing during the day or the night, respectively ($p \leq 0.01$ for both comparisons). Neither concentrate feeding, nor herbage allowance or stocking density affected N utilisation.

Conclusions: Daily pasture access, herbage botanical composition and supplement feeding are main factors influencing the N use efficiency of cows grazing temperate semi-natural grasslands. Part-time or full-time grazing of multi-species, semi-natural grasslands with restricted concentrate supplementation enables dairy cattle farmers to use home-grown feed protein sources at a moderate N use efficiency.

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49.

Consideration of body reserve change in modelling efficiency traits of dairy cows

Berücksichtigung der Körperreservenveränderung bei der Modellierung von Effizienzmerkmalen für Milchkühe

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Studies recommend the consideration of body reserve change (BRC) in efficiency traits to decouple the undesirable correlation between efficiency and energy balance (EB) in dairy cows. This study examines which body reserve trait and method is suited for considering BRC in calculating energy efficiency (EE).

Methods: Data were recorded in eleven German research institutes (Data: N = 50,472 weekly means; 35,754 Holstein [HO]; 14,718 Fleckvieh [FV]; 1,598 cows; data collection 2015-17)[1]. Traits describing BRC included daily change of body weight (dBW), body condition score (dBCS) and back fat thickness (dBFT). In method 1, the formula EE = MJ energy in milk/MJ NEL intake was adjusted to BRC as EEadj = (MJ energy in milk + b × MJ positive BRC)/(MJ NEL intake – b × MJ negative BRC)[2] using regression coefficients (b) for energy content of dBW, dBCS and dBFT from common literature (b-LIT)[3] and own estimates (b-OWN). Method 2 included dBW, dBCS or dBFT as covariable in the statistical analysis of EE. As main components the mixed model (SAS) included the fixed effects breed, parity (1, 2, 3+4, ≥5), lactation month (1-11), their interactions and in method 2 dBW, dBCS or dBFT as covariates. Pearson correlations showed the degree of decoupling between EE traits and EB.

Results: Influence of breed, parity, lactation month and their interactions on EE were mostly significant ($P<0.001$). Breeds did not differ utilizing dBFT. Main results of EE without considering BRC were 0.649 and 0.685 MJ lactation energy (LE)/MJ NEL for HO and FV, 0.117 MJ root mean square error (RSME), correlation (r) of –0.89 with EB. In both methods using dBCS resulted in lowest RSME (0.097-0.126 MJ LE/MJ NEL) followed by dBFT (0.102-0.140 MJ LE/MJ NEL) and dBW (0.110-0.187 MJ LE/MJ NEL). Including b-LIT caused a higher RMSE than b-OWN. Contrary to this, b-LIT mitigated the strong negative correlation between EEadj and EB more than b-OWN. EEadj with b-LIT for dBCS and dBFT correlated with EB between –0.62 and –0.74, while correlation using b-OWN ranged from –0.85 to –0.87. The stronger effect of b-LIT based on their consistently higher values compared to the estimates for b-OWN. The more BRC influenced EE, the flatter the EE lactation curve was. The apparently high EE in periods of mobilisation in early lactation decreased and the low EE in later lactation increased when body reserves were regained.

Conclusions: Considering BRC in calculating EE improved the negative relationship between EE and EB to a lower degree than expected and failed total decoupling. Regression coefficients for energy content of BRC estimated from own data set hardly affected correlation with EB. Additionally, they depend on data quality, on diverse possible estimation models and they reduce comparability of studies. Using common regression coefficients from literature requires reliable studies and data about body composition. General reasons like measurement accuracy, characteristics of body reserve traits or their effective ability to describe BRC completely have to be discussed. The results show, that considering BRC in calculating efficiency can be seen only as one step towards avoiding an apparently high efficiency based on increased mobilisation and on reduced regeneration of body tissue. Modelling efficiency requires a broad view on the animal, considering trade-offs between production and other life functions (e. g. health, fertility, longevity).

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50.

Comparison of the effect of bakery by-products with and without cocoa bean shells and a cereal concentrate on feed intake, reticular pH and milk production of herbage-fed dairy cows in early lactation

Vergleich des Effekts von Nebenprodukten der Backwarenindustrie mit und ohne Kakaoschalen und eines Getreidekraftfutters auf Futteraufnahme, retikulären pH und Milchproduktion von Gras gefütterten Milchkühen in der Frühlaktation

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Feeding human inedible or inappropriate resources, e.g. herbage and former food products, to ruminants is a key element for more sustainable livestock farming.Bakery by-products (BBP), rich in water-soluble carbohydrates (WSC), have been shown to be a suitable energy supplement for mid-lactating dairy cows fed total-mixed rations [1]. However, the use of BBP in herbage-based diets and for early-lactating cows has not yet been studied. Another source of by-products are cocoa bean shells (CBS). The CBS contain considerable amounts of bio-active polyphenols which may affect ruminal fermentation [2]. Therefore, the study aimed at evaluating if BBP and BBP containing cocoa bean shells can replace most of a cereal-based concentrate (CON) in a herbage-based diet fed to dairy cows in early lactation without negative consequences on the cows' production and ruminal fermentation.

Methods: We used 17 early lactating Holstein and Red Holstein cows (mean milk yield: 41 ± 7 kg/d, mean DIM: 36 ± 10 d). The study lasted for 6 weeks and included a baseline measurement (day before start of the study), a 2-wk adaptation (P1) and a 4-wk sampling period (P2). The cows were fed ad libitum freshly harvested herbage and were assigned to one of three CON types, balanced by milk yield, DIM and lactation number: i) a control CON (C, n=5), consisting mainly of maize (45%), barley (16%), rapeseed cake (9%) and oats (7%), ii) a CON consisting mainly of bakery by-products (55%), maize (31%), maize gluten (5%) and straw meal (5%) (BP-, n=6). In the third CON type, the straw meal of the latter CON was replaced by cocoa bean shells (BP+, n=6). The CON types were similar in protein and gross energy contents and were offered independently of the actual milk production according to a fixed allocation scheme from 5 kg in P1 to 6 kg in P2. Feed intake and milk yield were recorded daily, and milk composition twice weekly. Reticular pH was measured continuously (SmaXtec, Graz, Austria). Linear mixed models were run on ranked data and included the random effect cow and the fixed effects period, concentrate type, their interaction and – for feed intake – baseline herbage intake (R, package lme4).

Results: Herbage (16.2 ± 3.1 kg DM/d), CON intakes and consequently total DM intake (21.6 ± 3.1 kg) did not differ by concentrate type, except in P2, where C and BP- cows ingested more CON than BP+ cows (interaction: $p<0.05$). Across periods, C cows ingested less WSC (-0.9 kg) and more starch (+1.4 kg; both $p<0.05$) than BP- and BP+ cows, which was more pronounced in P2 than P1 (interaction: both $p<0.05$). Across periods, milk yield (37 ± 6.2 kg/d) and composition were not influenced by concentrate type (all $p>0.05$). Milk yield decreased from P1 to P2, which was less pronounced in C and BP- cows than in BP+ cows (interaction: $p<0.05$). In P2, milk lactose percentages were greater (4.9 %) in C cows than in BP- (4.7 %) and BP+ (4.6 %) cows. Across all periods, the range of the reticular pH of C cows was lower compared to BP- and BP+ cows ($p<0.05$). The mean, min and max pH increased from P1 to P2, which was less pronounced in C and BP+ cows than BP- cows (interaction: all $p <0.05$).

Conclusions: As expected, the inclusion of 55% of BBP in the concentrate resulted in an increased WSC and decreased starch intake. As reported earlier [3], the BBP-related greater WSC intake (BP- and BP+ cows) increased reticular pH levels, but also the pH range, whose relationship to rumen health is not yet conclusively studied. The lower concentrate intake and subsequently milk yield of cows supplemented by concentrate containing CBS needs further investigation regarding possible anti-nutritional factors of cocoa bean shells. The results need to be confirmed in future studies.

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51.

Faecal bacterial diversity and its relation to body weight and body composition of fattening pigs

Die bakterielle Diversität im Kot von Mastschweinen in Relation zur Körpermasse und Körperzusammensetzung

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Pig carcasses in EU member states are classified by body weight and lean meat content and paid according to classification. The majority of consumers prefer pork with low fat cover [1]. Current concepts of phase feeding are based on the average nutrient requirements of the animals. As pigs differ within the group in the level of feed intake and composition of growth, the slow-growing and fat-prone pigs could be fed more efficiently leading to a reduction in the use of resources without having a negative impact on performance. However, classifying and further subdividing pigs during the fattening phase is the prerequisite [2]. There are first indications that intestinal microbiota and porcine growth traits are linked [3]. Therefore, the aim of the study was to evaluate, whether faecal microbiota differ between pigs with differing body weight and body composition possibly reflecting varying feed intake and/or varying efficiency of nutrient utilization. Faecal microbiota analysis could be used to identify those pigs that ingest highest amounts of nutrients and/or best convert nutrients to growth or store the energy in a fat layer. Ultimately, feeding can be adapted to their requirements.

Methods: The study was performed in a conventional large group-housing barn (n=300 pigs) with automatic individual body weight recording. Feed and water was offered ad libitum. At four different times during the fattening period (26., 29., 31., 34. week of life), ultrasound examinations of the backfat and the M. longissimus dorsi were performed. Additionally, individual faecal samples of 40 pigs were collected for microbiota analyses (16S rRNA gene amplification within the hypervariable region V4, sequencing with Illumina MiSeq platform). Alpha diversity indices (Observed species, Shannon index) were measured in R (version 4.1.2) with the R-package "phyloseq" (version 1.36.0). For each measurement time, the 40 pigs were divided into two groups of equal size according to their body weight ("light" and "heavy") and subsequently categorized as "fat" or "lean" based on the calculated ratio of back fat thickness and muscle diameter. Alpha diversity indices were compared between the thus formed four groups ("light fat", "light lean", "heavy fat", and "heavy lean"). Statements of statistical significance were based upon p-values < 0.05.

Results: Alpha diversity increased with increasing age of the pigs and differed significantly between sampling time points (the 26th and29th towards the 31st and 34th week of life), which is why group comparisons were made between pigs within the first two and within the last two sampling time points. At any time, alpha diversity was the lowest in pigs classified as "heavy fat". However, bacterial richness and diversity did not differ significantly between the groups until week 29. In contrast from week 31 onwards, pairwise comparisons of bacterial diversity revealed significant differences between pigs classified as "heavy fat" and "light fat" ($p = 0.005$), and between pigs classified as "heavy fat" and "light lean" ($p = 0.017$). The present results indicate for a link between bacterial alpha diversity calculated in faecal samples and body composition/weight of pigs. In particular, bacterial diversity appears to be lower in heavy pigs with higher back fat thickness, whereas, differences to the other groups seem to be more pronounced with advanced age of the pigs. Nevertheless, no conclusion can be drawn as to whether the differences in alpha diversity are causally involved in the development of the body weight and composition. The bacterial diversity might be influenced, like growth and body composition itself, by other identical factors, such as the feed intake or an efficient ileal nutrient utilization with subsequent altered availability of substrates for microbial growth in distal parts of the intestinal tract.

Conclusions: The results suggest that faecal microbiota could be used as another indicator of the performance potential of fattening pigs.

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52.

Importance of genotype×feed interactions in nitrogen and phosphorus reduced rations of fattening pigs

Bedeutung von Genotyp×Futter-Interaktionen in Stickstoff- und Phosphor-reduzierten Rationen von Mastschweinen

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Strict governmental regulations coupled with high production costs are major reasons for nitrogen (N) and phosphorus (P) reduced rations for fattening pigs. The prerequisite for success of this feeding strategy is a high availability of nutrients in feed components, as well as efficent nutrient utilization of the pig. It is assumed that the genotype of an animal is the reason for variation in adaptability to dietary changes. The consequence is a re-ranking of genotypes: a genotype×feed-interaction (G×F). The aim of this study was to assess the effects of N- and P-reduced rations on N and P excretion and to investigate the existence of G×F.

Methods: 103 Pietrain×Large White crossbred boars (45) and sows (58), descending from 21 boars, were divided into one of two feeding groups, the control (C) or low-protein (LP) group at the start of fattening. Animals were individually housed and tested for performance in a 3-phase ad libitum fattening period until reaching a body weight of 115 kg. Diets were based on wheat, barley, triticale and the protein sources rapeseed meal and sunflower meal. According to the guideline for the implementation of strongly N and P reduced feeding procedures for pigs [1], C pigs were fed a reduced diet and LP pigs a strongly reduced diet. Faecal N and P excretion were calculated for each pig and fattening period from N and P intakes and digestibility, which was estimated from faecal spot samples using acid insoluble ash (AIA) as a marker. Feed and faecal samples were taken, with faeces collected twice daily for 5 days after a 7-day adaptation period. Feed and faeces were analysed for N, P and AIA. Urinary-N excretion in finisher I and II period was estimated via blood urea-N according to [2]. Statistical analysis was conducted using a linear mixed model with repeated measurements in R Studio. To determine the influence of fixed and random effects, an F-Test or a Likelihood Ratio Test was performed. The significance of the boar was quantified by estimating the heritability (h^2) and the proportionate variance of the interaction of boar×feeding group (gi^2). G×F was further investigated by ranking the boars based on their predicted values (rPD) in C and LP and on the corrected mean values of their offspring (roff) per group and fattening period. Corresponding rank correlations were calculated.

Results: There was a significant reduction (C vs. LP, $p<0.05$) in digestibility of N (finisher I: 79.8% vs. 77.2%, finisher II: 78.1% vs. 74.9%) and P (finisher I: 53.2% vs. 44.9%, finisher II: 57.4% vs. 52.9%) in LP in finisher I and II period. There were no differences in grower period. The urinary N excretion was significantly lower ($p<0.05$) in finisher I (23.0 g/d vs. 20.0 g/d). In finisher II, there was no difference. The estimated h^2 ranged from values close to zero for faecal P excretion to values of 0.56 for urinary N excretion. For all traits gi^2 was below 0.15, for urinary N-excretion it was 0.00. The influence of the boar and the interaction group was not significant ($p<0.05$). However, boar rank correlations indicate the existence of a G×F in some cases: in particular, the rPD for N and P excretion were 0.27 and 0.07 and thus clearly below the threshold of 0.80, which indicates the existence of a relevant G×F [3]. All other rPD and roff values were around or above 0.60. Hence, in these cases, a G×F could not be observed. The interpretation of h^2, gi^2, rPD and roff is limited in this study because of the comparatively small data set.

Conclusions: The results of the present study provide further evidence for the existence of G×F when fattening pigs are fed N and P reduced rations. Further analysis and validation with more data are needed.

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53.

Effects of dietary phosphorus and calcium supply and genetic background on expression of phosphate transporters in laying hens

Auswirkungen einer diätetischen Phosphor- und Calcium-Versorgung und des genetischen Hintergrunds auf die Expression von Phosphattransportern in Legehennen

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Phosphorus (P) and calcium (Ca) are essential nutrients involving in various biological processes. The egg laying period generates extra demand for maintaining cellular phosphate (Pi) homeostasis, which is potentially regulated by systemic regulators as well as its efflux across the cell membrane. Lohmann Selected Leghorn (LSL) and Lohmann Brown (LB) layer strains are most commonly used in commercial egg farming. Despite their identical egg production performance, these strains differ noticeably in other phenotypic traits leading to differences in their Pi needs [1]. Additionally, mineral Ca supply might have interfering effects on Pi absorption efficiency by formation of Ca-Pi complex [2]. According to the previous studies, the daily P intake and P excretion were significantly increased in LB and LSL hens fed with higher P concentration, while P utilization was lower in hens fed with high Ca concentration. However, Pi and Ca concentration in plasma did not significantly differ among the treatments and strains [1]. It was hypothesized that different strains of laying hens might cope with variations in mineral supply in different ways which could be reflected by the expression of Pi transporters in the small intestine and the liver. Besides sodium coupled Pi transporters, Xenotropic and Polytropic Retrovirus Receptor 1 (XPR1) has been shown to mediate Pi export from cells in mammals. Thereby, this protein is important for Pi homeostasis specially in metabolically active cells such as hepatocytes and enterocytes to provide a sufficient cellular Pi level [3].

Methods: 40 LSL and 40 LB laying hens were used in a study described before [1]. In brief, each of the strains was subdivided into four dietary treatments, standard dietary Ca concentration (39.6 g/kg dry matter; Ca+), reduced Ca concentration (33.9 g/kg DM; Ca-), standard dietary P concentration (5.3 g/kg DM; P+), reduced P concentration (4.7 g/kg DM; P-). Hens were fed one of the treatment diets for a period of 3 weeks, and then slaughtered. Liver tissue and jejunal mucosa were collected. Protein expression of Pi transporters (type IIb sodium phosphate cotransporter (Na-Pi IIb), type II Pi transporter (PiT2) and XPR1 in the enterocyte brush border membranes (BBM; XPR1 and PiT2 in liver) were semiquantified by Western blot. The data were analyzed by two-way analysis of variance (ANOVA) using Fit mixed model of JMP Pro software version 16. Significance was set at $p<0.05$.

Results: There was a clear strain effect in the expression of jejunal Pi transporters, indicating LB hens expressed more Na-Pi IIb, PiT2 and XPR1 in BBM than LSL hens ($p = 0.0004$, $p = 0.0002$ and $p<0001$, respectively). Interestingly, LB and LSL hens fed with less dietary Ca showed upregulation in Na-Pi IIb expression in BBM compared to the other dietary groups ($p<0.05$). Furthermore, low dietary Ca markedly upregulated the expression of XPR1 ($p<0.05$) in the liver of both strains, but the expression of PiT2 didn't change significantly.

Conclusions: In this experiment, strain effects were observed in expression of intestinal Pi transporters indicating LB hens have more intestinal Pi absorption efficiency, but this was not confirmed by P utilization [1]. However, Na-Pi IIb mRNA levels were also higher in LB hens [1]. In addition, expression of Pi transporters in the small intestinal mucosa and hepatic cells was inversely related to dietary Ca intake. Dietary Ca supply appeared to be a modulator of Pi transporters expression in the small intestine and liver; however, the functional meaning of this is unclear yet.

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54.

Stimulating saliva flow in sheep: Effects on variables of digestion and a comparison with cattle

Stimulierung des Speichelflusses bei Schafen: Auswirkungen auf die Verdauung und ein Vergleich mit Rindern

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High fluid turnover in the rumen has been shown to elevate the harvest of microbial yield from the forestomach. Microbial synthesis consumes metabolic hydrogen, competing with methanogenesis. Accordingly, in animals as well as *in vitro* continuous culture systems, high fluid turnover was linked to decreased methane yield [1, 2]. We had used pilocarpine as a saliva stimulant in cattle, and by this way decreased fluid retention in the total digestive tract by 7.8% and the methane yield per unit of digested dry matter by 6.5% [3]. In the present study the same pilocarpine dosages were applied in sheep, and the same variables were measured. The results were compared with those found in cattle to gain insights about ruminal physiology in dependence of saliva flow across ruminant species.

Methods: Three nonpregnant sheep (65-88 kg body weight [BW]) were used in a 3×3 Latin square design with three pilocarpine dosages (0, 2.5 and 5 mg/kg BW per day). They were fed hay (per kg dry matter [DM]: 142 g crude protein, 513 g neutral detergent fibre [aNDFom] and 10.0 MJ metabolizable energy) at around 85 g/kg$^{0.75}$ BW. The pilocarpine was given orally daily at 06:00, 14:00 and 22:00 h. Measurements included intake of feed and water, mean retention time (MRT) of fluid and particle (2-mm and 1-cm) in the reticulorumen (RR) and total gastrointestinal tract (GIT), rumen microbial yield (via urinary purine bases and metabolic faecal nitrogen), total tract methane emission (based on 48-h respiratory data), apparent nutrient digestibility (based on 7-days collection) and short chain fatty acids (SCFA) and pH of rumen fluid (obtained via oesophageal tubing). Data were statistically analysed in R for the presence of linear and quadratic effects using orthogonal polynomial contrasts.

Results: The MRT of fluid and small particles in the RR and total GIT, and the SCFA concentration in rumen fluid linearly declined with increasing pilocarpine dosage, while no quadratic relationship was detected. The MRT of fluid and small particles in the total GIT were decreased by 15% and 22%, respectively, when the highest pilocarpine dosage was used. Intake of feed and water, methane yield, apparent nutrient digestibility and microbial yield were not affected by pilocarpine. When comparing the sheep data with cattle data of [3], the ratio between particulate and fluid MRT in RR was smaller for sheep (1-cm particles and fluid: 2.13 for sheep and 3.27 for cattle; 2-mm particles and fluid: 1.74 for sheep and 2.58 for cattle); this was not affected by pilocarpine dosage.

Conclusions: The applied saliva stimulant increased the fluid flow rate in sheep, similar to what has been found in cattle; however, different from cattle, methane yield and metabolic faecal nitrogen did not respond to pilocarpine treatment in sheep. This discrepancy might be related to the difference between sheep and cattle in ratios of the MRT of particles to fluid.

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55.

Comparative analysis of pancreatic amylase activity in laboratory animal species

Vergleichende Analyse der Aktivität der Pankreas-Amylase bei Labortierspezies

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Pancreatic amylase is the enzyme for starch digestion acting in small intestinal chyme. Carbohydrates are usually the main source of energy in laboratory animal diets for maintenance. Thus, we aimed to get an overview of the pancreatic amylase activity of three common lab animal species to compare their digestive capacity for carbohydrates. Knowledge about such basic functions of digestive physiology are important to be able to choose the most suitable species for animal experiments.

Methods: Healthy laboratory animals which had not been in any other trial previously were used for the study: Eight-week-old C57BL/6J mice (n=11), Lewis rats (9 wks, n = 11), and RjHan:AURA hamsters (11 wks, n=23). All animals were fed the same commercial pelleted breeding diet for rats and mice at least 2 weeks prior to the analysis (22.7% crude protein, 5.1% crude fat, 4.6% crude fiber, 37.6% starch as fed; degree of starch gelatinization 34.5%). The animals were sacrificed and dissected. The pancreas was removed for analysis, as well as chyme from the anterior part of the duodenum. Amylase activity was tested using the Phadebas© method (Phadebas AB, Kristianstad, Sweden). The results were compared between species via Kruskal-Wallis test followed by Dunn´s Multiple Comparison test (significance level α=0.05; GraphPad Prism). In addition, chyme from several sites from the gastrointestinal tract was obtained. These samples were stained with Lugol´s iodine (starch staining) and evaluated via stereomicroscope.

Results: Hamsters (3885 ± 964 U/g pancreatic wet weight) showed a significantly lower amylase activity in pancreatic tissue than rats (9167 ± 5680 U/g) and mice (6281 ± 2493 U/g; $p<0.001$). In the duodenal chyme, the activity pattern was the same with hamsters having significantly lower values (496 ± 76 U/g duodenal chyme wet weight vs. 4027 ± 2367 U/g in rats and 6185 ± 1159 U/g in mice). The chyme samples from hamster forestomach showed a high amount of starch in the soluble phase, indicating the beginning degradation of starch granules. In the glandular stomach, the stained starch particles were more compact and the fluid phase was not stained.

Conclusions: The activity of pancreatic amylase was higher in rats and mice compared to hamsters. This finding is likely explained by hamsters having a forestomach where microbial fermentation of carbohydrates takes place, reducing the need for pancreatic amylase. Mice and rats seem to have a higher capacity for aut-enzymatic starch digestion by amylase. Hamsters have a non-glandular forestomach compartment in addition to the glandular part of the stomach. Structural and functional similarities of the hamster forestomach and the rumen of cattle have been shown [1,2]. If feed carbohydrates are fermented in the forestomach compartment of the hamster, less aut-enzymatic digestion (by host enzymes, as opposed to microbial fermentation) may be necessary. The microscopic images show the beginning degradation of starch in the hamster forestomach. All animals were young adult, so that the observed differences are not likely influenced by age. Species differences in digestive physiology need to be considered in selecting a laboratory animal species for a specific experiment. Further investigations comparing different age groups might be valuable to gain insight into the development of enzymatic activity.

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56.

Analysis of rumen specific fatty acids in hair of Holstein calves – a pilot study to assess the effect of age

Analyse von pansenspezifischen Fettsäuren im Haar von Holstein Kälbern – Eine Pilotstudie zur Beurteilung des Alterseffekts

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The identification of non-invasive biomarkers for assessing rumen development in calves are desirable. Odd and branched chain fatty acids in milk were shown as potential rumen-specific biomarker in dairy cows [1]. Since hair fat also contains odd chain, such as C17:0, and branched chain fatty acids, such as anteisoC17:0, it is unknown if rumen-specific fatty acids in hair are related to ruminal fermentation. Therefore, this pilot study investigated if rumen-specific fatty acids in hair alter with age.

Methods: Hair samples were taken from three 1-wk old calves (8 ± 1 days old; mean ± SD; no rumen digestion), three pre-weaned calves (93 ± 2 days old; significant rumen digestion) and three heifers (240 ± 6 days old; full rumen digestion). All calves and heifers belonged to the German Holstein breed and originated from the Research Farm of the FBN in Dummerstorf. Body weight was 42 ± 1.3 kg, 141 ± 3.8 kg, 324 ±4.5 kg for 1-wk old and pre-weaned calves and heifers, respectively. Calves were fed 8 L of colostrum and transition milk on day 1 and 2 of life. From day 3 until day 70, calves fed milk replacer up to 25 L/day. Weaning lasted from day 70 to day 102 of age. At the day of hair sampling, the daily milk replacer intake was 12.3 ± 0.6 L, 8.6 ± 2.8 L and 0 L for 1-wk old and pre-weaned calves and heifers, respectively. From birth on, calves had free access to concentrates and water. From day 14 of age, calves had also access to TMR. A hair sample was taken from the left side ventral of the foreleg from each calf and heifer. Hair lipids were extracted and analyzed from 200 mg cleaned and mill-ground hair using a fatty acid extraction kit [2]. After esterification and evaporation, fatty acid methyl esters were detected via gas chromatography. Fatty acids were expressed as relative values (%) of total reliably detected fatty acids. To evaluate if fatty acids in hair were influenced by age, groups were compared using the Kruskal-Wallis test. If a difference between the groups was found ($P < 0.05$), the Mann-Whitney U test was conducted as post-hoc test to determine which group differed from the other. Due to the small sample size, group differences were also reported as trends ($P < 0.1$).

Results: Thirty fatty acids were reliably detected in calf's hair. With special emphasis to rumen development, 14 odd and branched chain fatty acids and C18:1cis/trans-isomers were identified derived from rumen digestion. C17:0, anteisoC17:0 and C18:1*cis*-11 were different with respect to age or rumen development ($P < 0.05$). Contents of C17:0 and *anteiso*C17:0 decreased from 1-wk old (0.71 ±0.05%; 0.04±0.005%) to pre-weaned calves (0.37 ±0.02%; 0.02±0.004%; $P < 0.1$) and increased again in heifers (0.60 ±0.03%; 0.05±0.0006%; $P < 0.1$). Contents of C18:1*cis*-11 steadily increased from 1-wk old calves (1.1 ±0.1%) to pre-weaned calves (2.1 ±0.6%) to heifers (3.5 ±0.5%; $P < 0.1$).

Conclusions: Even if the sample size was small, this pilot study gives first evidence that rumen-specific fatty acids in calf's hair alter from no ruminant to ruminant digestion. Highest contents of C18:1*cis*-11 and *anteiso*C17:0 associated to heifers, while lowest contents of C18:1*cis*-11 were related to 1-wk old calves. The results need to be validated on the optimal time point of hair sampling to assess pure milk digestion in pre-weaned calves and verified by determination of solid feed intake and blood beta-hydroxybutyrate concentrations in blood plasma as a proxy for rumen digestion.

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57.

Role of different microbial groups in *in vitro* gas production from different tropical forages

Bedeutung verschiedener mikrobieller Gruppen für die in vitro Gasproduktion von verschiedenen tropischen Grundfuttern

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Tropical forage legumes and grasses vary considerably in their nutritive characteristics, such as crude protein (CP) and fiber concentrations and their digestibility. Different rumen microbial groups are involved in ruminal CP and fiber degradation. Thus, the aim of the present study was to have a better understanding of the role of different microbial groups in the *in vitro* gas production of different tropical forages and their mixtures.

Methods: This research evaluated one tropical forage legume i.e. Glycine max (L.) Merr (Soybean) from Brazil and one tropical grass i.e. Pennisetum purpureum Schumach (Elephant grass) from El Salvador. Proximate nutrient and fiber fractions of initial samples were analyzed[1]. The fiber digestibility of the initial forage samples was determined using the modified Tilley and Terry technique[2]. The forage Soybean and Elephant grass were incubated alone or in combination at the following legume:grass ratios (on dry matter; DM basis): 0:100, 30:70, 70:30, and 100:0. Each forage sample and their mixtures were incubated for 24 h using Hohenheim gas test with buffer solution in four different inocula prepared with different rumen fluids: original rumen fluid as well as rumen fluid without bacteria, rumen fluid without protozoa, and rumen fluid without fungi which were established by physical and chemical methods as described by Mobashar et al.[3]. Each forage sample and their mixtures were incubated in triplicate in three different runs. Blanks, hay standard, and concentrate standard were also prepared for 24 h incubation. The *in vitro* gas production after 24 h was analyzed by SAS 9.4 (SAS Institute Inc., Cary, NC, USA) using MIXED procedure considering legume proportion, type of rumen microbial inoculum, and their interactions as fixed effects. The effect of run was assumed to be random. The effects were declared significant at $P < 0.05$ Data were also tested for pairwise comparison using the SLICE statement when the ANOVA showed a significant for a significant interaction effect between legume proportion and type of rumen microbial inoculum.

Results: Calculated chemical composition and fiber digestibility varied greatly among forages and their mixtures. The concentrations of CP, amylase–treated, ash–corrected neutral detergent fiber (aNDFom), acid detergent fiber, and lignin of tropical forages and their mixtures ranged from 80 to 171 g/kg DM, 370 to 551 g/kg DM, 318 to 323 g/kg DM, and 32 to 82 g/kg DM, respectively. The concentration of undigested fiber did not vary among forages and their mixtures, meanwhile the potentially digestible neutral fiber concentration ranged from 119 to 303 g/kg DM. Fiber digestibility was lowest at a legume:grass ratio of 30:70 (119 g/kg aNDFom) and highest for Elephant grass (550 g/kg aNDFom). Irrespective of the forage source, the *in vitro* gas production was lowest when rumen fluid without bacteria was used as inoculum and highest with a buffered solution of the original rumen fluid ($P < 0.01$). The *in vitro* gas production was highest for the Elephant grass in all types of rumen microbial inoculum, but only declined linearly with increasing legume proportion with the inoculum containing rumen fluid without bacteria affected negatively by increasing legume inclusion in the diet ($P < 0.01$). The *in vitro* gas production decreased in the diets with legume proportion than legume:grass ratios 30:70 on DM basis.

Conclusions: Legume supplementation in the diets more than legume:grass ratios 30:70 on DM basis might reduce ruminal fermentation and gas production. Higher fiber concentration and digestibility lead to higher *in vitro* gas yield for grasses than forage legumes. Bacteria are decisive for fermentation, meanwhile limited contribution of fungi to overall gas yield is observed. Protozoa also contribute to fermentation of forage legumes.

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58.

In vitro digestion of plant and animal proteins in a canine model

In Vitro Verdauung von pflanzlichen und tierischen Proteinen am Hundemodell

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Due to animal well-being issues, environmental concerns, and ethical reasons, the demand for alternative protein sources to animal derived protein is increasing in pet nutrition [1]. Often, dog owners are feeding a plant-based diet rather than animal-based protein sources, but the nutritive evaluation and health impact is not fully known [2]. One important factor to consider is the protein digestibility [3]. The aim of the study was to investigate the protein digestibility in plant derived protein sources and compare it to the digestibility of animal protein.

Methods: For reasons of reproducibility and ease of execution, a static model was used for the *in vitro* experiment. The Infogest 2.0 method, originally designed to mimic human digestion, was adapted to the physiological digestion of the dogs' small intestine. Both animal (poultry, beef, offal, milk powder) and plant proteins (pea concentrate, pea isolate, soybean meal, soybean isolate) were used as substrates. To achieve a homogeneously mixed substrate during digestion, the substrates were ground to a particle size of 0.25 mm. The solubility of the protein source was considered to represent the digestibility. Different electrolyte stock solutions were prepared for the oral (OF), the gastral (GF) and the intestinal phase (IF) with different pH values (OF=pH 7.0; GF=pH 3.0, IF=pH 7.0). Within the intestinal phase, enzymes and bile salts were added to the solution. The gastral and intestinal digestion lasted for 2 hours each.

Results: Within the applied method, the solubility of the protein sources differed between plant and animal derived sources. Plant protein sources had a lower solubility and thereby a lower appreciated digestibility compared to animal derived proteins. Hence, the digestibility of soybean meal was estimated to 67.8 %, while it was 92.7 % for milk powder.

Conclusions: It was shown that the modified Infogest 2.0 method is suited to estimate the apparent digestibility of the used protein sources and that plant derived protein has a lower digestibility then animal protein sources.

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59.

Influence of oil carriers for feed oil on growth, crude fat digestibility and metabolisable energy in broiler chickens

Einfluss von Trägerstoffen für Futteröl auf Wachstum, Rohfettverdaulichkeit und Umsetzbare Energie bei Broilern

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Using carrier substances for fluids is an often-used strategy to achieve precise dosing and homogenous distribution of highly concentrated compounds. Carriers for feed oils may prospectively enable to supply liposoluble compounds more precisely but consequences of such carriers on the utilisation of the contained oil were not investigated to date. Inorganic carriers based on amorphous silica and lignocellulose-based carriers can be used for feed oils. The present study investigated whether growth, crude fat (CL) digestibility and nitrogen-corrected metabolisable energy (ME_N) in broiler chickens are affected by oil addition with and without an inorganic or organic carrier.

Methods: Six diets mainly containing maize, soybean meal and wheat gluten were mixed. The diets were formulated to meet or exceed recommendations [1] with 2% or 4% of refined rapeseed oil (low and high oil level, respectively). The oil was either added to the diets without a carrier, or included in 50:50 mixtures of oil and an inorganic carrier (Tixosil) or a lignocellulose-based carrier. Maize starch was included to the 2% rapeseed oil diets to achieve a similar calculated ME_N in all diets. Diamol was used to compensate for mass differences after addition of other ingredients to the diets. 780 unsexed Ross308 hatchlings were raised on litter receiving a commercial starter feed. On day 18, 15 birds each were moved to one of 48 metabolism units. The units were randomly assigned to the diets in a completely randomised block design with 8 replicates (blocks) per diet. Diets and water were available for ad libitum consumption throughout the experiment. Complete excreta were collected twice daily from days 24–27. The statistical model comprised carrier, oil level and the interaction as fixed effects, and blocks as a random effect.

Results: The carrier × oil level interaction was significant for average daily gain (ADG), average daily feed intake (ADFI), gain:feed ratio (G:F), digested CL, CL utilisation and MEN ($P \leq 0.022$). Lower ADG (67.4 vs 59.0 g/d), ADFI (99.5 vs 90.7 g/d), and G:F (0.677 vs 0.650 g/g) was observed for diets containing the inorganic carrier compared to diets without a carrier at the low oil level ($P < 0.001$). The oil level had no effect for the diets containing the inorganic carrier. Increasing the oil level increased ADG to 71.7 g/d ($P = 0.006$) and ADFI to 105 g/d ($P = 0.002$) when no carrier was added. In diets containing the lignocellulose-based carrier, increasing the oil level raised ADG (66.8 vs 70.2 g/d; $P = 0.025$) and G:F (0.682 vs 0.707 g/g; $P < 0.001$). At the low oil level, ~4.9 g CL/d were digested without carrier effect. At the high oil level, 7.7 g CL/d was digested. Adding the lignocellulose-based and inorganic carrier reduced digested CL to 6.8 g/d ($P = 0.023$) and 6.4 g/d ($P = 0.007$), respectively. The CL utilisation was greater for the high than for the low oil level ($P < 0.001$). The lignocellulose-based carrier did not affect CL utilisation (79% and 83% at the low and high oil level, respectively) while the inorganic carrier increased CL utilisation by 2 %-units and 3%-units at the low and high oil level, respectively ($P \leq 0.003$). The MEN was not different among all treatments (15.5–15.8 MJ/kg dry matter), except for a higher MEN when the lignocellulose-based carrier was added at the high oil level (16.0 MJ/kg dry matter; $P = 0.005$).

Conclusions: The results of this study do not indicate adverse effects of the lignocellulose-based carrier for feed oils on growth and CL utilisation, but it increased ME_N. This may have been connected with an increased G:F. Hence, possible benefits of carriers related to precise dosing and homogenous distribution of highly concentrated compounds may be achieved without drawbacks in CL utilisation and ME_N. The reduced growth of broilers fed inorganic carrier may have been a consequence of high oil-binding capacity of the carrier, which was not fully used by the oil addition and caused the feed to absorb moisture.

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60.

Uptake of L-methionine and 3-O-methyl-D-glucose in different intestinal regions of broiler chickens

Aufnahme von L-Methionin und 3-O-Methyl-D-Glucose in verschiedenen Darmregionen von Masthühnern

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Methionine (Met) as the first limiting amino acid in poultry has a key role in the performance of broiler chickens [1]. Met, other amino acids and glucose are absorbed via specific transport systems, including Na^+-dependent and Na^+-independent transporters [1, 2]. It is widely accepted that the small intestine has the highest absorptive capacity for amino acids and monosaccharides in mammals and chickens; however, contrary to mammals, chicken show nutrient absorptive capacity along the whole intestinal tract, including large intestinal regions [3]. The aim of our study was to elucidate the functional uptake capacity of L-Met and 3-O-methyl-D-glucose (3-OMG) in duodenum (DUO), jejunum (JEJ) and caecum (CAE) of broiler chickens.

Methods: A total of 30 male and 23 female day-old chickens (Cobb 500) were fed an adapted starter diet for three weeks, followed by a finisher diet for at least 10 d. Thereafter, chickens were slaughtered. Isolated epithelial preparations from duodenum, middle jejunum and caecum were used for apical uptake studies. Uptakes were measured at a final mucosal concentration of 50 µM or 5 mM of [^{14}C]-L-Met and [^{3}H]-3-OMG in Ussing chambers in the presence or absence of mucosal sodium. All buffered solutions (pH 7.4 ± 0.03) were gassed with 95% O_2/5% CO_2. Data were analyzed using two-way ANOVA with factors intestinal segment and sodium, and their two-way interaction. Data are presented as least square means with SEM.

Results: At 50 µM substrate concentration, uptakes of L-Met and 3-OMG were affected by intestinal segment, sodium and intestinal segment × sodium interaction ($P < 0.01$ each). Further dissection of the intestinal segment × sodium interaction for L-Met identified no difference of uptakes in DUO and CAE irrespective of the presence (DUO, 0.19 ± 0.03; CAE, 0.14 ± 0.03 nmol·cm^{-2}·min^{-1}) or absence of Na^+ (DUO, 0.17 ± 0.03; CAE 0.14 ± 0.03 nmol·cm^{-2}·min^{-1}) ($P > 0.05$). Similarly, dissection of the intestinal segment × sodium interaction for 3-OMG identified no difference of uptakes in DUO and CAE irrespective of the presence (DUO, 0.14 ± 0.02; CAE, 0.14 ± 0.02 nmol·cm^{-2}·min^{-1}) or absence of Na^+ (DUO, 0.13 ± 0.02; CAE 0.15 ± 0.02 nmol·cm^{-2}·min^{-1}) ($P > 0.05$). Of note, uptakes in JEJ were greater than uptakes in DUO and CAE ($P < 0.05$) with an additional difference for uptakes in the presence vs. absence of mucosal sodium for both L-Met (0.76 ± 0.03 vs. 0.57 ± 0.03 nmol·cm^{-2}·min^{-1}; $P < 0.05$) and 3-OMG (0.49 ± 0.02 vs. 0.31 ± 0.02 nmol·cm^{-2}·min^{-1}; $P < 0.05$). At a mucosal concentration of 5 mM, uptakes of L-Met were only affected by tissue ($P < 0.01$) with highest uptakes in JEJ ($P < 0.05$; data not shown). By contrast, uptakes at 5 mM 3-OMG were affected by intestinal segment, sodium and intestinal segment × sodium interaction ($P < 0.01$ each). There were no differences of uptakes in DUO and CAE ($P < 0.05$; data not shown). However, uptakes in JEJ were again higher than uptakes in DUO and CAE and higher in the presence vs. absence of sodium (40.7 ± 1.4 vs. 27.6 ± 1.4 nmol·cm^{-2}·min^{-1}; $P < 0.05$).

Conclusions: The present results suggest that JEJ is the main intestinal segment for amino acid and glucose absorption in broiler chickens. Despite proposed nutrient uptake capacity, DUO and CAE appear to have minor contribution to the uptake of tested nutrients under the applied experimental conditions. The disappearance of sodium dependence of jejunal L-Met uptake at a mucosal concentration of 5 mM points to the fact that Km value(s) of the involved transporter(s) is markedly lower than 5 mM [1].

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61.

Effects of TNF and Cannabidiol on porcine intestinal IPEC-J2 cells

Effekte von TNF und Cannabidiol auf IPEC-J2-Darmzellen des Schweins

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In recent years cannabidiol (CBD) gained major attention in manyfold studies, because of its anti-inflammatory, anti-convulsing and analgetic effects without being psychotropic. CBD, extracted from Cannabis sativa, can exert its effect by binding to cannabinoid receptors [1]. Beneficial effects of CBD have already been demonstrated in different models, but it remains unknown whether these effects also occur in porcine enterocytes. Recently it has been shown that proinflammatory cytokine Tumor Necrosis Factor alpha (TNFα)-treated porcine jejunal epithelial IPEC-J2 cells serve as an appropriate inflammatory model of the intestinal epithelium [2,3], showing a disturbed barrier function. Therefore, this study was performed to investigate whether CBD can prevent the barrier weakening effect of TNFα, using IPEC-J2 cells.

Methods: IPEC-J2 cells were grown on semipermeable cell culture inserts until confluency.For analyzing the CBD effects under inflammatory conditions, 1000 U/ml TNFα was added to the basolateral compartment, and different concentrations of CBD were added apically. To investigate the epithelial barrier function, the transepithelial resistance was measured for 48 h, employing an epithelial volt-ohm meter. Statistical analysis was performed using JMP 16 software. Data were compared by using Kruskal-Wallis test and statistical significance was determined by Dunnett's post hoc test. Statistical significance was assumed at values below $p = 0.05$.

Results: Sole incubation with1000 U/ml TNFα led to a significant decrease in transepithelial resistance over 48 h compared to controls (TNF: 69.64 ± 2.76 %; ctrl: 89.89 ± 2.01 %; $^{***}p < 0.001$, $n = 16$). 40 µM CBD mitigated this effect induced by TNFα (TNF + 40 µM CBD: 84.72 ± 4.58 %; $^{**}p < 0.01$, $n= 16$). Thus, there was no significant difference observed between controls and TNF / 40 µM CBD treated cells after 48 h.

Conclusions: Our study reveals that CBD can prevent a decrease of transepithelial resistance induced by TNF, indicating a beneficial effect under inflammatory conditions. Further investigations to identify possible effects on intestinal epithelial barrier proteins as an underlying molecular mechanism are in progress.

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62.

Carbohydrate oxidation patterns in pigs fed high and low fiber diets as determined by a naturally ^{13}C enriched test meal

Kohlenhydratoxidation bei Schweinen, die mit faserreichem oder faserarmem Futter gefüttert wurden, unter Verwendung einer natürlich mit ^{13}C angereicherten Testmahlzeit

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Carbohydrate oxidation can be measured using tracer methodologies. The "gold standard" is the ^{13}C-breath test, which determines ^{13}C recovery in breath CO_2, after consumption of a ^{13}C enriched test meal [1]. Chemically ^{13}C labeled nutrients are highly enriched but expensive, so nutrients with a higher natural ^{13}C enrichment, C4 plants (e.g. corn) vs. C3 plants (e.g. wheat), can be used. Natural ^{13}C enrichment is expressed as delta values (‰ $\delta^{13}C$) and ^{13}C in corn-derived nutrients is ~8–16 ‰ more abundant ($\Delta\delta$) than in C3 plants (i.e. wheat) [2]. Carbon dioxide derived from nutrient oxidation is not only present in breath but can also be captured in blood or saliva. Therefore, the carbohydrate oxidation pattern of a cornflakes test meal was measured by breath ^{13}C enrichment and compared to alternative CO_2 sources, in pigs adapted to high (HF) or low (LF) fiber diets.

Methods: Eighteen male piglets born to multiparous (parity 2 – 8) German Landrace sows, with litter sizes of 12-20 piglets were selected. Piglets were weaned at 28 days of age and fed a standard post-weaning diet ($\delta^{13}C$ -28.7 ‰). At 50 days of age, piglets were allocated to HF (n = 9; 6.5% crude fiber, $\delta^{13}C$ -25.7 ‰) or LF (n = 9; 2.8% crude fiber, $\delta^{13}C$ -25.6 ‰) diets, based on bodyweight (BW) and sow, and adapted to their diets for 3 days (25, 50, 75%) until 100% (experimental day 1; ED1). The HF and LF diets were fed isoenergetically and twice daily (07:30; 16:30h). Drinking water was provided ad libitum. Feed intake was recorded daily and BW was measured every Monday and Thursday, to calculate feed conversion ratio (FCR). Faecal score was recorded at ED-4, -3, 4, 5, 11, 12 and 16. At ED18, pigs were fitted with a jugular catheter, and at ED22, pigs were transferred to individual metabolic cages and fed a meal consisting of 3 g of cornflakes/kg BW (Kellogg's; $\delta^{13}C$ -14.1 ‰), mixed with 10% of their experimental feed. Saliva, breath and blood samples were taken at -30, -15 (basal), 60, 120, 180, 240, 300, 360, 420 and 480 min post-feeding. Isotope-ratio mass spectrometry was used to measure $^{13}CO_2$ enrichment in feed, breath, blood, RBC (red blood cells) and saliva. Area under the enrichment-time-curve (AUC), maximum enrichment (E_{max}) and time to maximum enrichment (t_{max}) were calculated by best curve fit (TableCurve 2Dv5.01). Data was analysed using the mixed model of SAS, with repeated measures where required.

Results: No difference in BW, feed intake, FCR or faecal score was observed between HF and LF piglets ($p > 0.05$). In HF pigs, the $\Delta\delta$ $^{13}CO_2$ enrichment was higher than in LF pigs, in breath from 120 to 480 min ($p < 0.05$), saliva, at 180 ($p < 0.01$) and 300 min ($p < 0.05$), and in blood at 180 and 480 min ($p < 0.05$). Breath (979 vs. 703 ‰ · min; $p < 0.01$) and blood (841 vs. 536 ‰ · min, $p < 0.05$) AUC was greater in HF than LF pigs. Breath (2.9 vs. 2.2 ‰, $p < 0.01$) and saliva (2.8 vs. 2.1 ‰, $p < 0.05$) E_{max} was higher in HF than LF piglets. Breath (221 vs. 222 min), blood (212 vs. 196 min), RBC (238 vs. 214 min) and saliva (214 vs. 210 min) t_{max} was not different ($p > 0.05$) between HF and LF pigs, respectively.

Conclusions: The higher ^{13}C Emax and AUC values in HF pigs than LF might be due to a faster digesta passage rate in HF pigs. Breath appears to be a more sensitive and reliable sample medium compared to blood, RBC and saliva when ^{13}C enrichment values of the test meal are low. However, saliva could have potential as a non-invasive sample alternative to the minimally invasive breath test.

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63.

The impact of fibre source and fibre particle size on performance and nutrient digestibility in growing pigs

Der Einfluss der Faserquelle und Faserpartikelgröße auf die Leistung und Nährstoffverdaulichkeit in wachsenden Schweinen

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Dietary fibre is mainly classified according to its chemical characteristics as soluble and insoluble fibre in different solvents. As it is known from some species, the structure and particle size of fibre-rich feedstuff is also decisive when digestive features and performance is evaluated [1]. So far, this was investigated only in a few studies in pigs. It was the aim of the study to compare the impact of coarse and finely ground dried hemp plants and apple pomace on the nutrient digestibility and weight of digestive organs in growing pigs.

Methods: Apple pomace and dried hemp plants were added to the diet of growing pigs either coarse or finely ground to reach neutral detergent fibre (NDF) levels of 25.5 % in the diet. The experimental diets were based on corn and soybean meal and contained titanium dioxide as indigestible marker for the solid phase. To determine the impact of the experimental diets on performance and protein digestibility, 54 healthy nine weeks old barrows and gilts (DanBred x Duroc) were allotted randomly to 28 pens (2 piglets per pen, 7 pens per treatment) according to their body weight. The piglets received the experimental diets for three weeks and feed intake and individual body weight was recorded weekly to calculate the feed conversion ratio (FCR) and bodyweight gain (BWG). Eight pigs per group were sacrificed and ileum and rectum contents were collected. The weight of the stomach, jejunum, ileum, caecum, colon, liver and pancreas was determined as well as the gut filling. The stomach mucosa was scored for lesions. Apparent ileal (AID) and total tract (ATTD) protein, amino acids (arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, valine), calcium, phosphorous, copper and zinc digestibility/disappearance were calculated. Statistical analyses were performed using the software SPSS (IBM, version 25, USA). Two-way ANOVA with Tukey-HSD post hoc test were used to determine statistical differences ($p < 0.05$) and to form subgroups. The animal trial was approved by the Regional Office for Health and Social Affairs Berlin: LaGeSo StN 023/21.

Results: Regarding the stomach score, no differences were observed between fibre sources ($p = 0.729$) or fibre particle size ($p = 0.421$). Significant differences in feed intake (fibre source x particle size interaction, $p = 0.004$), bodyweight gain (fibre particle size, $p = 0.018$; fibre source x particle size interaction, $p = 0.040$), and feed conversion ratio (fibre particle size, $p = 0.012$) were observed between feeding groups. Here, the fine fibre particle size showed the highest BWG and lowest FCR. The relative pancreas ($p = 0.045$), stomach ($p < 0.001$), and jejunum ($p = 0.010$) weights were higher in animals fed diets containing apple pomace and a corresponding trend was observed for the ileum ($p = 0.099$). In contrast, the relative liver, caecum and colon weights were not affected by fibre source or particle size. While no differences in AID and ATTD protein digestibility was observed. Precaecal disappearance of phosphorous was significantly affected by fibre particle size ($p = 0.035$), while the disappearance of calcium and zinc was influenced by fibre source ($p < 0.001$; $p = 0.016$). AID of all investigated amino acids was significantly higher in animals fed larger fibre particle sizes, while ATTD was affected by both fibre source (arginine, isoleucine, leucine, phenylalanine, valine) and fibre particle size (arginine, histidine, lysine, methionine, phenylalanine, valine).

Conclusions: Chemical, as well as physical parameters of the main fibre component in the diet influenced performance and nutrient digestibility in growing pigs. Thus, our results suggest that it is not sufficient to evaluate and report chemical characteristics of the dietary fibre source, the structure must be considered in a future fibre evaluation system.

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64.

Effect of increasing soluble-dietary-fibre content in diets of fattening pigs on growth performance

Einfluss eines steigenden Gehalts an „soluble-dietary-fibre" in Rationen von Mastschweinen auf die Wachstumsleistung

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Fibre is an important component of diets for pigs. Negative effects (e.g. dilution of energy concentration) are well known, but also many positive effects (e.g. increased satiety or SCFA production) have been demonstrated [1]. Fibre is a highly complex feed constituent making its definition and measurement difficult. Several analytical methods are used in animal nutrition to characterize fibre (e.g. crude fibre, detergent-fibre analysis), but these methods do not quantify the fraction of soluble fibres (e.g. pectins). The total-dietary-fibre (TDF) analysis quantifies a major part of the soluble fibre fraction and differentiates between soluble and insoluble dietary fibre (SDF, IDF). The aim of the present study was to investigate the effects of increasing SDF concentrations in diets for fattening pigs, while keeping the TDF concentration, energy and protein supply constant. We hypothesized that at equal TDF concentration more energy is released by fermentation of SDF, supporting higher average daily gain (ADG), without impairing the feed conversion ratio (FCR).

Methods: A fattening trial was conducted with 60 OEHYB piglets starting with Ø 45 kg (±2.6). Animals were kept in 12 pens à 5 animals in a conventional fattening barn equipped with automated feeding troughs allowing individual feed intake (FI) measurements on daily basis. Animals were weighted weekly. Four diets of dry feed (treatments, T1-T4) were fed ad libitum in 2 phases (45-79 kg (±6.3), 79 kg-slaughter (±6.7)), nutrient concentrations were in accordance with recommendations of GfE [3]..The diets differed only in the percentage of SDF of the TDF content, which was 150 g/kg for all treatments. The percentage of SDF in TDF increased from 3% in T1, 7% in T2, 11% in T3 to 15% in T4. This change in soluble fibre content was attained by adding sugar beet pulp in exchange of lignocellulose. All other nutrients were kept constant for all diets. Performance data were analysed at 77 days in trial (experimental period). Two animals (T1, T4) reached their final slaughter weight earlier and were excluded from the data set and another animal (T2) was excluded due to health reasons. The experimental design was a block design with 4 treatments and 3 blocks. A mixed-model analysis was performed with SAS© Studio with block as a random effect and "age at 77 days in trial" and "starting weight" were considered as covariables.

Results: At the end of the experimental period the weight of the animals was 107.4, 107.7, 111.0 and 113.1 kg for T1-T4. The ADG in this period was 845.4 g (±77.37) in T1 and 839.0 g (±116.84) in T2 and increased in T3 and T4 to 893.7 g (±97.53) and 920.9 g (±75.68). T1 and T2 were different ($p<0.05$) from T4, but not from T3, which did not differ from T4 either. The FI was 182.0 kg (±16.52) and 184.0 kg (±24.61) in T1 and T2 for the experimental period. There was a tendency ($p<0.1$) for increased FI in T3 and T4 with 197.7 kg (±28.13) and 198.0 kg (±29.17). No difference was found for the FCR, which was 2.82, 2.88, 2.90 and 2.81 for T1-T4.

Conclusions: Reasonable fattening performances were achieved for all treatments, however the treatments with higher amounts of SDF had higher ADG due to increased FI. The increased FI may result from an increased microbial activity in the hindgut of fattening pigs due to higher amounts of soluble and easily fermentable fibre, leading to faster degradation of the digesta and therewith faster passage through the GIT (van Leeuwen & Jansman, 2007). Another explanation can be an increased FI due to better taste of the diets containing higher amounts of sugar beet pulp (T1 0%, T2 1%, T3 5%; T4 10%). Additional measurements (e.g. digesta SCFA, histomorphology) will allow a comprehensive data interpretation in future. The FCR was not different between treatments, suggesting that the increased intake of soluble fibre up to 15% SDF of TDF did not affect the nutrient use efficiency. The chosen level of TDF allowed reasonable growth performances in the presented trial.

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65.

Does 25-hydroxyvitamin D_3 have any advantages in respect to mineral homeostasis, bone metabolism and lameness of pigs fed protein- and phosphorus-reduced diets in comparison to conventional vitamin D_3?

Hat 25-Hydroxyvitamin D_3 gegenüber konventionellem Vitamin D_3 Vorteile in Bezug auf den Mineralstoffhaushalt, den Knochenstoffwechsel und die Fundamentgesundheit bei restriktiv mit Protein und Phosphor gefütterten Schweinen?

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Nutrient-reduced diets, which are used to reduce emissions, can negatively affect mineral homeostasis if not properly balanced or confected, and thus increase the risk of lameness if the animals are challenged by additional factors like gastrointestinal diseases or suboptimal housing conditions. Dietary supplementation of the vitamin D metabolite 25-hydroxycholecalciferol (25-OHD_3) has been shown to increase vitamin D status more efficiently than administering vitamin D_3 [1]. For sows, we have recently reported a reduction of gait changes when animals were supplemented with 25-OHD_3 instead of vitamin D_3 [2]. The present study aims at comparing the effects of the two vitamin D metabolites on the prevalence of gait changes, lesions, and lameness in rearing and fattening pigs.

Methods: The study was carried out with the offspring of sows from our former study [2] that were either supplemented with 25-OHD_3 or conventional vitamin D_3 (50 µg/kg feed each). The animals (Topigs 20 × Pietráin) were assigned to four treatments: Piglets from vitamin D_3 sows that received a creep diet (N = 384; 15.9% CP, 0.65% P) supplemented with 50 µg vitamin D_3/kg feed (VD/VD) and later on a respective rearing (N = 384; I: 15.8% CP, 0.52% P; II: 16.2% CP, 0.52% P; III: 15.7% CP, 0.47% P) and fattening diet (N = 288; I: 15.5% CP, 0.46% P; II: 14.7% CP, 0.41% P; III: 13.8% CP, 0.39% P), piglets from vitamin D_3 sows supplemented with 50 µg 25-OHD_3/kg feed (VD/25D), piglets from 25-OHD_3 sows supplemented with 50 µg vitamin D_3/kg feed (25D/VD) and piglets from 25-OHD_3 sows supplemented with 50 µg 25-OHD_3/kg feed (25D/25D). The legs were examined for swelling (carpal/tarsal joints) using a three-stage score (0 = no swelling; 1 = lesion < 2 cm; 2 = lesion ≥ 2 cm) at the age of 6 wk, 8 wk, 10 wk, 14 wk, 18 wk, 20 wk, and 22 wk). To assess the gait changes, each animal was monitored on a 16 m walk according to the following key: 0 = no gait changes; 1 = movement not fluid (asymmetrical walking), 2 = mild lameness, step is shortened, reduced weight-bearing on affected limb; 3 = severe lameness, minimum weight-bearing on affected one limb. Fisher's exact test was used to analyze the qualitative parameters. When $P < 0.05$, the null hypothesis of no significant differences between the control and experimental treatments was rejected.

Results: Piglets from sows supplemented with 25-OHD_3 (25D/25D, 25D/VD) presented with a lower prevalence of leg swellings during rearing compared with VD/25D (6 wk, -18%) and VD/VD (6 wk, -27%; 8 wk, -22%; 10 wk, -22%). The direct supplementation of the offspring with 25-OHD_3 instead of vitamin D_3 was not associated with the prevalence of leg swellings during rearing. During fattening, a positive effect of 25-OHD_3 fed to the sow and to the offspring could only be observed at the age of 14 wk, when the highest prevalence of leg swellings was found in VD/VD pigs (56%) and the lowest in the 25D/25D group (15%). At the age of 6 wk, we also observed a significantly lower prevalence of gait changes in the 25D/VD group (8%) in comparison to VD/VD piglets (21%). Direct supplementation of the offspring with 25-OHD_3 seemed to reduce the prevalence of gait changes at the age of 14 wk (25D/25D, 15% vs. 25D/VD, 34%), 20 wk (25D/25D, 32% and VD/25D, 42% vs. VD/VD, 63%) and 22 wk (25D/25D, 24% and VD/25D, 18% vs. 25D/VD, 46% and VD/VD, 46%). Severe lameness was not observed at any time during this trial.

Conclusions: Leg swellings can be a hint for more time spent lying. The lower prevalence of leg swellings and gait changes might be explained by an effect of the increased plasma concentrations of 25-OHD_3 in the 25D groups on muscle function. Studies done in humans and rodents have indicated a direct role of vitamin D metabolites on muscle cell differentiation and contractility [3]. Furthermore, we have already observed an increase in standing time after feeding in sows supplemented with 25-OHD_3 [2].

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66.

Effects of dietary calcium source, acidification, and phytase on precaecal phytate degradation and phosphorus digestibility in pigs

Einfluss der Calciumquelle im Futter, Ansäuerung und Phytase auf den praecaecalen Phytatabbau und die Phosphorverdaulichkeit beim Schwein

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Limestone ($CaCO_3$) is a common Ca source in pig diets with a high acid binding capacity, potentially increasing stomach pH and reducing precaecal (pc) myo-inositol hexakisphosphate ($InsP_6$) degradation and thereby P digestibility. Replacing $CaCO_3$ by Ca formate, or chemical acidification might avoid increase of stomach pH. Possible interactions between exogenous phytase and Ca sources on $InsP_6$ degradation and mineral digestibility in growing pigs have not been studied. Hence, the aim was to assess the effect of different Ca sources ($CaCO_3$ vs. Ca formate), chemical acidification, and exogenous phytase supplementation on $InsP_6$ degradation, P and Ca digestibility in pigs.

Methods: A basal diet based on maize (58.6%), soybean meal (25%), and rapeseed meal (10%) was mixed with either 1.0% $CaCO_3$ or 1.1% Ca formate, without or with exogenous phytase (hybrid 6-phytase at 1,500 FTU/kg). A fifth diet was formulated by adding 8 g/kg formic acid to diet $CaCO_3$ with phytase. Titanium dioxide was included as indigestible marker and diamol to balance mass differences for all diets. Eight barrows (initial body weight of 24.1 ± 1.7 kg were fitted with a simple T-cannula at the distal ileum and assigned to the five dietary treatments in a completely randomized row-column design. Daily feed allowance was 4% of mean body weight of all pigs. The experiment included five periods of 10 days each composed of five days of diet adaption, followed by three days of faeces and urine collection, and two days of ileal digesta collection. Data were analysed in a nested two-factorial analysis of variance using ProcMixed of SAS 9.4. Considered fixed effects were acidification (yes and no), Ca source ($CaCO_3$ and Ca formate) within acidification and phytase supplementation (0 and 1,500 FTU) within acidification. A one factorial analysis of variance of all diets (ProcMixed) was used to compare CaCO3 with phytase to chemically acidified CaCO3 with phytase. Multiple t-test was used for pairwise comparison of treatment means.

Results: All diets contained 4.3 g P/kg and 2.4 g $InsP_6$-P/kg. There were no significant differences between diets in the pc digestibility of dry matter, gross energy and crude protein. The pc $InsP_6$ disappearance and P digestibility in pigs were greater in diets with phytase compared to without phytase ($P < 0.001$). However, pc $InsP_6$ disappearance and P digestibility was lower in Ca formate diets compared to $CaCO_3$ diets ($P \leq 0.032$). Exogenous phytase supplementation resulted in lower ileal $InsP_5$ and $InsP_4$ concentrations for the $CaCO_3$ diet compared to Ca formate diet ($P \leq 0.019$). $InsP_3$ and $InsP_2$ isomers were detected in the ileum when diets contained phytase and concentrations did not differ between the Ca sources ($P > 0.05$). The myo-inositol concentration in the ileum was greater in diets with phytase than without ($P < 0.001$) but not affected by Ca source ($P > 0.05$). The acidification of the $CaCO_3$ with phytase diet resulted in greater pc $InsP_6$ disappearance and pc P digestibility compared to $CaCO_3$ with phytase diet (87 vs. 80% and 55 vs. 61%; $P \leq 0.027$). The $InsP_4$ and $InsP_3$ concentration in the ileum showed no differences between the two diets($P > 0.05$), however ileal concentration of $InsP_5$ isomers were lower and ileal $InsP_2$ and myo-inositol concentration was greater upon acidification ($P \leq 0.031$). The pc digested Ca was greater for diets with phytase than without ($P < 0.001$).

Conclusions: The results suggest that Ca formate is no alternative to $CaCO_3$ in diets for pigs when high $InsP_6$ degradation is intended. The decreased release in InsP-P might be explained by the higher solubility of Ca formate which might lead to a faster and higher concentration of free Ca^{2+} complexing with InsP isomers in the anterior digestive tract. However, the release of InsP-P by exogenous phytase in $CaCO_3$ diets can still be further increased by chemical acidification. This might be explained by a decrease in gastric pH which supported the enzymatic activity of the used phytase.

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67.

Trace mineral concentrations in the liver of growing Fleckvieh bulls

Die Konzentration an Spurenelementen in der Leber wachsender Fleckviehbullen

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Bovine liver is a part of human nutrition but is also considered to be a health issue for humans due to its high copper content. Since the performance potential of Fleckvieh (German Simmental) fattening bulls has been improved by selective breeding during the past decades, the animals' liver trace mineral concentrations might have changed. Hence, a feeding experiment ending with a serial slaughter trial was conducted to evaluate the trace mineral concentrations in the livers of growing Fleckvieh bulls.

Methods: 72 Fleckvieh bulls (age: 42 d, body weight (BW) 80 kg) were fed restricted amounts of milk replacer (120 g/l) and a concentrates/hay-based total mixed ration (TMR) until weaning at an average BW of 121 kg and subsequently on a TMR based on maize silage and concentrates for ad libitum intake. The fattening period began at an average BW of 225 kg. Bulls were randomly allocated to normal energy (NE) and high energy (HE) treatment groups fed 11.6 and 12.4 MJ ME/kg DM, respectively. Differences in the TMRs' energy concentrations were achieved by varying the percentage of maize silage and concentrates in the rations. The feed trace mineral concentrations were kept constant in relation to feed dry matter. Hence, feed trace mineral content did not differ between NE and HE groups. Individual feed intake was recorded daily, and BW was determined at four-week intervals. The bulls were slaughtered in five final live weight groups of 120 (n=8), 200 (n=10), 400 (n=18), 600 (n=18), and 780 kg (n=18). During slaughter, the animals' livers were removed, weighed, homogenized, and chemically analyzed for their trace mineral concentrations (Fe, Cu, Zn, Mn). Statistical analysis was performed using Proc Mixed of SAS (Version 9.4). The analysis included a two-way ANOVA with interaction (feed energy, weight group, feed energy x weight group). Results are shown in ranges and standard error of means and were compared by the PDIFF option with values of $p<0.05$ regarded as significant.

Results: Increasing the amount of concentrate in the HE-ration resulted in significantly greater daily trace mineral intake of HE bulls. Despite the higher trace mineral intake, no differences in trace mineral concentrations were observed in the livers of HE and NE fed bulls. The liver weight increased from 1.9 to 8.8 kg in bulls with 120-780 kg live weight. Liver Fe, Zn, and Mn concentrations increased from the lowest to the highest weight group and ranged between 40.1-46.7 mg/kg ±0.92, 34.4-37.3 mg/kg ±0.56, and 2.1-2.6 mg/kg liver ±0.04, respectively ($p<0.05$). Contrary, liver Cu concentration decreased during growth from 125.7 to 84.4 mg/kg liver in bulls with 120-780 kg live weight ($p<0.05$).

Conclusions: Results on high liver Cu concentration in calves confirm observations by the Federal Office of Consumer Protection and Food Safety, which specified the Cu concentration in calf liver to be 141 mg/kg liver [1]. The Commission Regulation (EC) No 149/2008 declares the maximum residue level of Cu in the bovine liver to be 30 mg/kg liver weight [2]. Our results demonstrate that this recommendation is exceeded even in the liver of bulls with 780 kg live weight, which showed the lowest liver Cu concentration in this study. The EFSA [3] recently re-evaluated the Cu intake in humans and recommended the maximum Cu intake to be 0.07 mg per kg body weight per day (5 mg/day in adults). Based on our findings, this daily dose is reached by consuming 40 g calf liver or 59 g beef liver, but no adverse health effects are expected with infrequent consumption of bovine liver.

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68.

Impact of dietary lithium on female fecundity in *Drosophila melanogaster*

Einfluss von diätetischem Lithium auf die weibliche Fruchtbarkeit von Drosophila melanogaster

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The alkali metal lithium receives growing attention in nutritional research although its exact molecular actions are not yet fully established. Recent reports suggest that lithium may stimulate stem cell proliferation. In the present study we investigated the role of dietary lithium on fecundity in *Drosophila melanogaster* which is a suitable experimental model to study nutrient regulation of ovarian function. After mating, the adult female fruit fly continuously produces oocytes from germline stem cells, a process that is partially regulated by insulin signaling and glycogen synthase kinase-3 (GSK-3). Both are crucial for glucose metabolism and oogenesis which are highly evolutionary conserved among species and described to be targeted by lithium. Hence, we decided to examine whether dietary lithium modulates oogenesis in D. melanogaster.

Methods: The *D. melanogaster* strain w1118 was cultured at 25°C, in a 12 h light/dark cycle at 65% humidity. Mated female flies aged 2-3 days were treated with a standard sugar-yeast diet (5% sugar/10% yeast/2% agar) supplemented with 0, 0.1, 1 or 5 mM LiCl. The daily egg production was quantified over the following 20 days of dietary intervention (n=12-22, originating from three individual approaches). Feed intake was quantified to account for possible differences in the nutritional status of the experimental groups (n=13-14, repeated once). In addition, the developmental performance of the F1 generation was monitored (n=12-15 vials collected from three approaches at day five of the dietary intervention). At 1 mM LiCl, the impact of lithium on differential gene expression in *Drosophila* ovaries was analyzed by RNA sequencing (n=5, comprising 15 ovaries each). Protein expression was captured by Western blotting (n=4, comprising 15 ovaries each) and immunohistochemistry. Differences in the formation of glycogen storages during late oogenesis was analyzed in stage 10 and stage 14 follicles using Periodic acid Schiff reaction (PAS) (n=23-52, originating from two approaches).

Results: Dietary supplementation studies revealed that nutritive to supra-therapeutic lithium doses of 0.1 up to 5 mM LiCl increase life time egg production in the fruit fly. The largest effect compared to the non-supplemented control was observed at 1 mM LiCl by up to 45 %. The feed intake remained largely unchanged in all groups. In this regard, it is remarkable that a relatively low nutritive concentration of lithium (0.1 mM LiCl) was already sufficient to significantly change the transcript level of about 400 genes in the *Drosophila* ovaries. Furthermore, maternal lithium treatment does not affect developmental ability of the F1 generation indicating the absence of teratogenic effects. Western blot analysis revealed that lithium administration only slightly increases levels of phosphorylated/inactivate GSK-3 in the ovary. Accordingly, glycogen staining of the oocytes showed no difference between lithium-treated (1 mM LiCl) and control flies.

Conclusions: To our knowledge, the improved fecundity is the first phenotype to be observed at a nutritive lithium dose in *Drosophila melanogaster*. It is likely that systemic effects of lithium account for the increased fecundity. Hence, future studies should investigate the involvement of other tissues and the corresponding molecular mechanisms.

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69.

Effects of phytase dosage and dietary phytate concentration on the precaecal phytate disappearance and phosphorus digestibility in broiler chickens

Effekte von Phytasedosierung und Phytatkonzentration im Futter auf das praecaecale Phytat-Verschwinden und die praecaecale Phosphor-Verdaulichkeit bei Broilern

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In plant-based feed, the major storage form of phosphorus (P) is phytic acid ($InsP_6$) and its salt phytate. Hydrolysing enzymes such as phytase are needed to release P and myo-inositol from $InsP_6$. When fed diets supplemented with 1500 phytase units (FTU)/kg containing varying $InsP_6$ concentrations coming from different oilseed meals, a linear increase in precaecal (pc) $InsP_6$ disappearance per added phytase relative to the dietary $InsP_6$ concentration was found in broilers [1]. Therefore, this study investigated the relationship between dietary $InsP_6$ concentration and phytase supplementation on pc $InsP_6$ disappearance, P digestibility, and myo-inositol concentration in ileal digesta.

Methods: The experiment was arranged in a 4×3-factorial design with 4 dietary $InsP_6$ concentrations (5.6, 7.6, 9.7 and 11.7 g/kg dry matter (DM)) and 3 phytase dosages (500, 1500, and 3000 FTU/kg of a 6-phytase, Natuphos E). The $InsP_6$ concentration was increased by substituting maize starch by a 50/20/20/10 mixture of soybean meal, rapeseed meal, sunflower meal, and rice bran, which concurrently increased dietary P, calcium and crude protein (3.5 to 7.3, 5.9 to 7.7 and 187 to 341 g/kg DM), respectively). No mineral P was added to the diets and TiO_2 was used as an indigestible marker. Until 14 days of age, male broilers received a commercial starter diet. On day 14, 840 broilers were moved to 84 metabolism units with 10 animals each and units were arranged in a completely randomised block design with 7 replicates (units) per diet. On day 22 and 23, digesta from the terminal small intestine were collected and pooled on a pen basis. Feed and digesta were analysed for $InsP_6$, total P, myo-inositol, and TiO_2. Results were analysed by a two-way ANOVA using the MIXED procedure of SAS 9.4.

Results: The $InsP_6$×phytase interaction was significant for pc $InsP_6$ disappearance (P=0.007), pc P digestibility (P=0.001), and the myo-inositol concentration in the ileal digesta (P=0.006). The $InsP_6$ disappearance and P digestibility values linearly decreased with increasing dietary $InsP_6$ from 83 to 56% and from 80 to 62%, respectively (P≤0.022) at 500 FTU/kg. $InsP_6$ disappearance was not different within $InsP_6$ concentrations when 1500 and 3000 FTU/kg were used but decreased slightly with increasing dietary $InsP_6$ (91 to 83%; P≤0.046). Precaecal P digestibility was not different within $InsP_6$ concentrations between 1500 and 3000 FTU/kg, except for a higher P digestibility at 2.1 g $InsP_6$/kg and 3000 FTU/kg (80 vs 85%; P≤0.024). At 1500 FTU/kg, P digestibility decreased from 86 to 80% when $InsP_6$ was increased from 1.6 to 2.1g/kg DM and remained on this level at higher $InsP_6$ levels. P digestibility at 3000 FTU/kg was unaffected when $InsP_6$ was increased from 1.6 to 2.7 g/kg DM (~86%) and then decreased to 81% at 3.3 g/kg DM. Myo-inositol concentration in ileal digesta at 500 FTU/kg was 25.2 µmol/g DM and not affected by dietary $InsP_6$. At 1500 FTU/kg, myo-inositol was not different between 1.6 and 2.1 g $InsP_6$/kg DM (28.3 µmol/g DM) and increased to 39.2 µmol/g DM at 3.3 g $InsP_6$/kg DM (P≤0.001). At 3000 FTU/kg, myo-inositol increased with increasing dietary $InsP_6$ from 28.2 to 39.6 µmol/g DM until 2.7 g $InsP_6$/kg DM (P≤0.019) and remained at this level at 3.3 g $InsP_6$/kg DM. When $InsP_6$ disappearance was related to FTU dosage, a linear relationship with dietary $InsP_6$ was found at all phytase dosages, with slopes of linear regressions indicating an $InsP_6$ disappearance of 0.376, 0.276, and 0.144 µmol/100 FTU at 500, 1500, and 3000 FTU/kg, respectively.

Conclusions: The linear relationships between $InsP_6$ disappearance per added phytase and dietary $InsP_6$ concentration showed that the efficiency of supplemented phytase did not depend on dietary $InsP_6$ concentration. The inverse relationship between $InsP_6$ disappearance and ileal myo-inositol concentration indicated that phytase effects on the complete dephosphorylation of $InsP_6$ also was influenced by phytase dosage but not dietary $InsP_6$ concentration.

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70.

Effect of phosphorous availability in malted wheat on bone mass and bone mineral status of a slow-growing growing broiler breed (Ranger Classic)

Einfluss der Phosphorverfügbarkeit in gemälztem Weizen auf Knochenmasse und Mineralstoffgehalt im Knochen einer langsamwachsenden Broiler Herkunft (Ranger Classic)

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Up to 80% of the phosphorous (P) contained in wheat is stored as phytic acid. Phytic acid is an anti-nutritive substance, which chelates cations like iron, copper and zinc, thereby reducing their availability [1]. Usually, the exogenous phytase of microbial origin is used to improve phosphorus and trace mineral utilization, but this is prohibited in European organic production systems. Lemmens et al. [2] showed in their study that germination and malting is a proper tool to denature the phytate P in wheat kernels by activating plant phytases embedded in the seeds. Therefore, this study investigated the effect of malted and non-malted wheat in combination with varying dietary phosphorus (P) levels on bone mineral status of slowly growing broiler chickens under organic farming conditions.

Methods: Four different diets were formulated for this trial containing either wheat or malted wheat (dried using 55°C recirculating air) in combination with and without P supplementation from 1.5% monocalcium-phosphate (0.3% P vs. 0.4% P in diet), respectively. 200 1-day-old broiler chickens (Ranger Classic) were used for this experiment. From d1 to d16 the birds were fed with a commercial organic starter diet. On d17, an equal number of birds from both sexes were randomly allocated to the four experimental diets (5 pens per diet, 10 birds per pen) comprising a completely randomized design. On d57, birds were slaughtered and both tarsometatarsus bones of each bird were taken, pooled within each pen and the weight of the pooled sample was recorded. Bone dry matter (DM) (103°C, 48h) and ash (550°C, 6h) were determined and P in bones was analyzed photometrically and calcium (Ca), magnesium (Mg) manganese (Mn), iron (Fe) copper (Cu) and zinc (Zn) using Inductively Coupled Plasma Mass Spectrometry (ICP-MS). Statistical analyzes comprised two- way ANOVA (wheat variant, P-level and interaction).

Results: P supplementation caused higher bone weight ($P<0.01$), bone DM ($P<0.01$) and higher bone ash ($P<0.01$). The wheat variant had no significant influence on these parameters. In contrast, P supply had no significant influence on P and Mg status of bones. However, birds fed with wheat had significantly more P and Mg in bone ash ($P=0.003$; $P=0.012$), than birds fed with malted wheat. Furthermore, P supplementation reduced concentrations of Zn, Fe and Mn in bone ash ($P= 0.044$; $P=0.004$; $P<0.001$), but no significant interactions were found.

Conclusions: P supply improved bone weight, bone DM and bone ash, which had probably a positive effect on bone density, but did not affect the mineral composition of bone, nor zootechnical response [3]. The present study showed that malting did not improve P and trace mineral utilization under given experimental conditions.

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71.

Microbiome of lambs influenced by feeding system and algae blend supplementation

Mikrobiom von Lämmern beeinflusst durch Fütterungssystem und Algenmischung

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Lambs raised on an extensive system, fed on pasture have healthier meat than those raised on intensive systems, fed on concentrate [1]. Recent interest has emerged on algae as alternative and sustainable ingredients for ruminant feeding. Microalgae and seaweed are valuable sources of macro- and micronutrients and bioactive compounds with potential to promote meat quality and consumers' health. Although studies assessed the effects of dietary inclusion of individual microalgae or seaweed species on lamb growth performance, the combined effects of microalgae and seaweed supplementation on gut microbiota has not yet been unveiled. This study aimed to evaluate the effect of microalgae and seaweed blend supplementation to concentrate fed lambs on gut microbial communities from rumen, abomasum and colon.

Methods: Three groups of ten Bordaleira lambs were assigned to one of three diets: pasture, commercial concentrate (concentrate) and concentrate supplemented with 5% (w/w, dry matter basis) commercial microalgae and seaweed blend (Algaessence®; Allmicroalgae and AlgaPlus, Portugal) (algae). Animals had free access to meadow hay and fresh drinking water. After 60 days, lambs were slaughtered in a commercial slaughterhouse and gut digesta from rumen, abomasum, and colon collected. DNA was extracted with a commercial kit and sequence targeting bacterial and archaeal communities. Raw reads were demultiplexed and analyzed in Qiime2 [2]. Taxonomies were assigned using the Silva database. Alpha diversity was measured by Faith's phylogenetic diversity and Shannon's entropy, and beta diversity represented by Bray-Curtis distances. For statistical analyses, the Kruskal-Wallis H-test and PERMANOVA were used. Differentially abundant taxa were detected by ALDEX2 [3].

Results: Despite the differences found among rumen, abomasum and colon populations, many microbial genera showed similar behavior within diets. Statistical analysis of archaeal and bacterial profiles revealed that among all diets pasture was significantly different from others. Archaeal genera Methanobrevibacter and Methanomethylophilus yielded higher abundances in concentrate and algae diets compared to pasture, while members of Methanomethylophilaceae were more represented in the latter ($P<0.1$). Among bacteria, genera Butyrivibrio, Clostridia, Pseudobutyrivibrio, Quinella, Succiniclasticum and Saccharimonas were mostly represented in pasture ($P<0.1$). In concentrate and algae diets, abundances of Acetitomaculum, Bifidobacterium, Limosilactobacillus, Ruminobacter, Succinivibrio, Blautia, Anaerostipes, Coprococcus and Dialister were higher compared to pasture ($P<0.1$). Alpha diversity metrics of animals fed on pasture were higher than those fed with other diets for archaeal and bacterial communities and at all sampling sites ($P<0.05$). No differences in alpha diversity between concentrate and algae diets were observed for all sampling groups. Regarding beta diversity, pasture samples were grouped as separate clusters in both bacteria and archaeal analysis. PERMANOVA analysis revealed that for archaeal samples at all sampling sites pasture was significantly different from other diets ($P<0.05$), with no differences between concentrate and algae. At the same time, bacterial Bray-Curtis distances were different between all diets for all sampling sites ($P<0.05$).

Conclusions: Pasture diet resulted in different microbial communities and composition compared to the concentrate and algae diets at all sampling sites. Compared to concentrate, algae supplementation resulted only in differences in bacterial beta diversity, while alpha diversity metrics and archaeal beta diversity were not affected.

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72.

Inverse relationship between microbial growth and CH_4 yield in grass and corn silages measured via Hohenheim Gas Test

Negative Korrelation zwischen Mikrobenwachstum und Methanbildung in Gras- und Maissilagen gemessen über Hohenheimer Futterwerttest

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Theoretically, microbial synthesis competes with methanogenesis in consuming metabolic hydrogen. The efficiency of microbial synthesis was suggested to negatively correlate with short chain fatty acid (SCFA) concentration [1], and an inverse relationship between microbial yield and produced gas volume was reported using a modified Hohenheim Gas Test (HGT) [2]. The latter study defined the partitioning factor (PF) as a measure of the variation of fermented end products (gas/SCFA) per unit degraded substrate, with a higher PF indicating more degraded substrate was directed towards the synthesis of microbial biomass. Based on twenty-three silage samples incubated in HGT, we aimed to investigate the relationship between microbial and CH_4 yield, and to evaluate the potential of the PF as the index of how much degraded substrate was directed to microbial yield or fermented end products.

Methods: Fifteen grass silages (173 ± 31.1 g crude protein [CP]/kg dry matter [DM], 42.5 ± 6.31 g ether extracts [EE]/kg DM, 521 ± 49.3 g neutral detergent fibre [aNDFom]/kg DM and 9.9 ± 0.84 MJ metabolizable energy [ME]/kg DM) and eight corn silages (73.5 ± 7.21 g CP/kg DM, 28.6 ± 4.19 g EE/kg DM, 408 ± 24.3 g aNDFom/kg DM and 10.8 ± 0.33 MJ ME/kg DM) were incubated in HGT syringes for 24 h (about 200 mg DM; 6 replicates per sample, distributed over 6 rounds as single incubations). The produced gas volume was recorded and sampled for CH_4 measurement. The substrate residues were collected by centrifugation, and the resulting pellet was weighed to estimate the apparent organic matter (OM) degradability. In a replicated syringe, the substrate residues were collected in Gerhardt fibre bags and boiled in neutral-detergent solution to estimate the true OM degradability (TOMD). The microbial mass was considered as the difference between the apparently and truly degraded substrate. The initial microbial mass before incubation was measured in the same way in the inocula. The net increase of microbial biomass production was calculated as the microbial mass at the end of fermentation minus the initial microbial biomass. The PF was calculated as ratio of truly degraded OM/total gas production. The data were analyzed in R (package "lme4") using linear mixed model with silage type as random factor, to investigate the correlation among CH_4 yield, net microbial increase and PF, and the effects of initial microbial mass and TOMD on CH_4 yield and net microbial increase.

Results: The corrected 24-h gas production was 44.6 ± 6.3 mL/200 mg DM for grass silage and 54.5 ± 2.1 for corn silage. The net microbial increase correlated negatively with CH_4 yield ($P<0.001$). As expected due to its definition, the PF correlated positively with net microbial increase ($P<0.001$) and negatively with CH_4 yield ($P<0.001$). The net microbial increase correlated positively with TOMD ($P<0.001$), but negatively with initial microbial mass ($P<0.001$). The CH_4 yield correlated positively with TOMD ($P<0.001$) and with initial microbial mass ($P<0.001$).

Conclusions: This study gives support to the concept of a negative correlation between microbial growth and methane production. The partitioning factor in fact indicated how much degraded substrate was directed to microbial synthesis or fermented end products. Interestingly, a lower initial microbial mass was associated with an increase in microbial synthesis and lower methane production, which might relate to the microbial growth stage.

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73.

MicroRNAs in plasma and leucocytes as potential biomarkers for rumen health in a SARA cow model

MicroRNAs als potenzielle Biomarker für die Pansengesundheit in einem SARA-Kuhmodell

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MicroRNAs (miRNAs) are small non-coding RNAs which are crucial regulators of gene expression and could serve as biomarkers for several biological conditions. Sub-acute ruminal acidosis (SARA) is a metabolic disorder marked by low ruminal pH, and has been linked to the feeding of starch-rich diets. Early biomarkers are urgently needed to identify cows that suffer from SARA, since there are no clear clinical signs, but subsequently a drop in milk yield. We hypothesize that circulating miRNAs in blood could serve as potential biomarkers to detect cows with SARA. To test it, a comprehensive identification of miRNA profiles in plasma and leucocytes was conducted and miRNAs were evaluated as potential biomarkers in cows in which SARA was induced through high-grain feeding.

Methods: Cows (N = 5) were fed a diet of 100% forage (FOR) and were transitioned with a one week adaption period to a 65% high-grain diet (HG), which was fed for a total of four weeks. The FOR diet consisted of 75% grass silage, 15% corn silage, and 10% hay (17.2% crude protein, 50.4% NDF and 4.2% starch). The HG diet included 26.3% grass silage, 8.7% corn silage and 65% concentrate to provide 19.5% crude protein, 31.0% NDF and 28.5% starch based on DM. DMI increased after the switch from FOR to HG diet (8.43 kg vs. 13.31 kg). Cell-free plasma and blood leucocytes were obtained using a two-step centrifugation protocol. Total RNA was isolated and small RNA libraries were prepared of samples from FOR and one week HG diet. A 50 bp single end sequencing approach was performed on an Illumina NovaSeq 6000 platform at CeGat (Tübingen, Germany). Differentially expressed (DE) miRNAs were considered with a false discovery rate (FDR) ≤ 0.05 and a $|\log2 \text{ (fold change)}| \geq 1$. Small RNA-qPCR was done from FOR, one week HG and 3 weeks HG diet samples utilizing a poly-A-technique (miRNA 1st-Strand cDNA Synthesis Kit, Agilent Technologies). MiRNA-specific primers were designed and validated. MiR-107 and miR-103 served as reference miRNAs to normalize for RNA content. Relative expression was calculated using the ddCT-method. Relative values were calculated relative to the mean expression in FOR.

Results: In plasma and leucocytes, a total of 520 and 730 miRNAs were found, respectively. From these, 498 miRNAs were found to be shared by plasma and leucocytes, with 22 being expressed solely in plasma and 232 being exclusively found in leucocytes. Sixty-three circulating miRNAs in plasma were found only in cows fed with high-grain diet, showing that these animals had a higher circulating miRNA count and diversity. The differential expression analysis identified 10 up-regulated and 2 down-regulated miRNAs in plasma. Candidate biomarkers were chosen for further analyses based on total read counts of filtered and sorted miRNAs expressed in all cows fed a high-grain diet, as well as differentially expressed miRNAs in terms of their read count and log2 fold change. These miRNAs were then queried against the available literature, as well as the annotated functions on miRBase 22. The best candidates were bta-miR-11982, bta-miR-1388-5p, bta-miR-1306, bta-miR-12034, bta-miR2285u, and bta-miR-30b-3p. Small RT-qPCR validation included a further sampling time point, when the cows had already been fed HG for three weeks. A Kruskal-Walli's test showed a strong influence ($p = 0.035$) of the high-grain diet on the expression of miR-2285u. Furthermore, we found a higher expression of miR-30b-3p in week one on HG ($p = 0.098$), compared to the forage feeding. The same direction of effect was detected within the NGS data.

Conclusions: Our findings show that dietary modifications affect the release and expression of miRNAs in systemic circulation, potentially modulating post-transcriptional gene expression in SARA-affected cows. MicroRNAs bta-miR-2285 and bta-miR-30-3p in blood plasma appear to be promising candidates to serve as biomarker for SARA and will be further analyzed.

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74.

Effect of physically effective fiber concentration on rumen fermentation, digesta passage rate, and nitrogen metabolism of lactating cows

Einfluss des physikalisch effektiven Fasergehaltes auf Pansenfermentation, Passage des Verdauungsbreis und Stickstoff-Stoffwechsel von laktierenden Milchkühen

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The aim of the study was to evaluate the effect of increasing physically effective fiber (peNDF) concentration of a total mixed ration (TMR) on rumen microbial protein synthesis (MPS) and fermentation, solid and liquid passage rates, and performance of lactating dairy cows, which consequently will alter partitioning in their nitrogen (N) excretion.

Methods: Four lactating rumen-fistulated Holstein cows with an average (mean ± standard deviation) milk yield of 31.9 ± 2.69 kg/d and 75 ± 8.4 days in milk (DIM) were assigned to a 4 x 4 Latin Square consisting of four periods of 21 d with 13 d of adaptation and 8 d of sampling. The TMR was composed of corn silage, grass silage, grass haylage, barley straw, concentrate and mineral mixtures, ground corn grain and soybean extraction meal. Cows were offered one of four TMR which were identical in their chemical composition. Diets were formulated to have a negative rumen N balance (RNB; – 2.1 g/kg dry matter). The peNDF concentration of the TMR was adjusted by varying its mixing time: 15, 30, 45, and 60 min, corresponding to peNDF concentrations (particles >8.0 mm) of 202, 208, 221, and 238 g/kg dry matter. Data were analyzed by PROC MIXED (SAS V9.4) with peNDF and period as main effects, DIM as covariable, and animal as random factor and tested for linear and quadratic contrasts.

Results: Increasing peNDF concentration affects quadratically nutrient intakes ($P \leq 0.03$), apparent total tract digestibility of organic matter ($P=0.06$), and MPS ($P=0.02$) with lower values for the high and low peNDF than both medium peNDF diets. Also, eating and total chewing times were longer for both medium peNDF diets ($P \leq 0.02$), while there was no difference in rumination time ($P \geq 0.28$) across diets (all in min/d). Milk yield, milk fat-to-protein ratio, and urea-N did not differ between diets ($P \geq 0.10$). Both liquid and solid digesta passage rates were not affected by mixing time ($P \geq 0.37$). The rumen pH was similar across diet ($P \geq 0.88$), but the molar proportions of acetate decreased linearly ($P=0.03$) and of propionate increased ($P \leq 0.01$) linearly with increasing mixing time. Increasing peNDF concentration affected quadratically partitioning of N excretion, with a lower proportion of the ingested N excreted via urine ($P<0.01$) and a greater proportion secreted via milk ($P<0.01$) for the high and low peNDF than both medium peNDF diets.

Conclusions: Results indicate that feeding dairy cows a negative RNB diet with varying peNDF concentrations adjusted by TMR mixing time affects their intake, digestibility, and N metabolism which suggests a need for a better understanding of the effect of negative RNB as affected by peNDF concentration.

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75.

A blend of botanicals can reduce LPS-induced inflammation and disruptive effects on apical-out chicken and porcine enteroids

Eine Mischung aus pflanzlichen Stoffen kann LPS-induzierte Entzündungen und schädigende Effekte bei Enteroiden von Huhn und Schwein mit außenliegender apikaler Membran reduzieren

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In vitro studies about the efficacy of feed additives to fight intestinal inflammation or pathogens are usually performed on 2D primary enterocyte monolayers or immortalized cells [1]. Enterocyte monolayers are useful but they lack the different epithelial components, which are essential to better study the various properties of botanicals. For this purpose, intestinal organoids could be a valid alternative. Nash et al. (2021) published a study about the deep characterization of chicken apical-out enteroids [2] and recently, Joo et al. (2022) demonstrated that by sub-culturing basal-out pig enteroids on ultra-low attachment plates it is possible to obtain porcine apical-out enteroids [3], which are both ideal for *in vitro* functional studies. The aim of this study was to investigate the ability of a blend (BOT) containing thymol and other botanicals, to reduce the disruptive effects of LPS-induced inflammation on apical-out chicken and porcine enteroids.

Methods: To obtain chicken enteroids, intestines of 18 day-old chicken embryos were recovered and digested with collagenase type I. The digested tissue was filtered through cell-strainers to recover the >40 μm fraction. The obtained cell aggregates were then cultured in suspension for 4 days with a proper organoid floating medium to generate mature chicken enteroids. To obtain pig enteroids, intestinal crypts were obtained from 35 days-old pigs' jejunum using EDTA. Recovered intestinal crypts were cultured embedded in extracellular matrix for 2 days to allow the formation of basal-out organoids. Then, on day 3 basal-out enteroids were recovered and cultured for 24 h in suspension on ultra-low attachment plates to generate apical-out organoids. Mature enteroids of both species were then challenged with lipopolysaccharide (LPS) for 6 h. In total, three different groups were present: buffer control, LPS control and a treated group (LPS+BOT). FD4 paracellular permeability (PCP) and gene expression for selected markers were then evaluated.

Results: Chicken enteroids responded to the LPS challenge by showing a 60% increase in FD4 PCP, a near 60-fold increase in IL1β expression, a 4-fold increase in IL6, and IL8 levels ($p<0.01$), a 7-fold increase in tumor necrosis factor-α (TNFα) expression ($p<0.01$) and a 30-fold increase in interferon-γ (INFγ - $p<0.01$) expression. Moreover, a decrease in zonula occludens 1 (ZO1 - $p<0.05$), occludin (OCCL - $p<0.05$), and toll-like receptor 4 levels were observed ($p<0.01$). BOT showed anti-inflammatory properties in reducing all the effects connected to the LPS challenge. FD4 PCP was decreased by 40% ($p<0.05$), and the expression of all pro-inflammatory markers was significantly reduced compared to the challenged control. BOT restored the ZO1 and OCCL levels near the buffer levels ($p<0.05$). Moreover, BOT significantly increased the defensin beta 4a (DEFB4A) expression over the negative control ($p<0.05$). This could be an interesting effect to improve the defense ability of the host against pathogens. Furthermore, apical-out porcine enteroids also well responded to the inflammatory challenge by showing a 70% increase in FD4 PCP, a 2-fold increase in IL1β, IL8, and mucin 4 (MUC4) levels ($p<0.01$), and a 3-fold increase in IL6 expression ($p<0.01$). Moreover, a decrease in ZO1 and occludin OCCL levels was observed. BOT showed beneficial properties in reducing all the effects connected to the LPS challenge. FD4 PCP was decreased by 35% ($p<0.05$), and IL1β, IL6, IL8, and MUC4 expression were significantly reduced compared to the LPS control. Lastly, BOT increased the ZO1 and OCCL levels by 50% ($p<0.05$), acting as a barrier-reinforcing agent.

Conclusions: BOT showed interesting anti-inflammatory properties both on chicken and pig enteroids, being able to maintain epithelial integrity thus reducing LPS-induced damage *in vitro*. This thymol-based blend has the potential to be further investigated as a feed additive to improve chickens and pigs ability to overcome stressful phases during their lifecycle.

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76.

Assessing the effects of dietary probiotics on gut barrier and immune-related gene expression, histomorphology, and growth in broilers with or without pathogen challenge

Beurteilung der Effekte von Probiotika auf die Expression von Genen für die Barriere und Immunantwort im Darm, Darmstruktur und Wachstumsleistung bei nicht-infizierten und infizierten Broilern

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Probiotics have been proposed to improve the gastrointestinal health of broilers, but data in the literature are inconsistent. Using a meta-analytical approach, the present study aimed to quantify relationships between published data for dietary probiotics and gene expression related to gut barrier and immune functions in broilers challenged with or without pathogens. We further examined the effect of dietary probiotics on gut histomorphology and growth parameters.

Methods: From the 54 included scientific journal articles published between 2012 and 2022, individual data for gut barrier and immune-related gene expression, histomorphology, and growth were extracted. Subsets of data were built and separately analyzed for trials with pathogen or without pathogen challenge. Prediction models were parameterized on the effect of probiotics which consisted of *Bacillus*, *Lactobacillus*, *Clostridium*, *Enterococcus*, *Bifidobacterium*, *Paenibacillus*, *Pediococcus*, and *Saccharomyces*, accounting for inter- and intra-study variability using PROC MIXED and REG in SAS. All measured variables were expressed in fold change values between probiotic and control treatments.

Results: The means of dietary probiotic levels for non-pathogen and pathogen groups were 2.69 x 10^5 CFU/kg and 2.14 x 10^5 CFU/kg, respectively. In the non-pathogenic groups, positive relationships could be established between probiotics and fold-change values of barrier-related gene expression in small intestine at weeks 3 or 6 of life, which included jejunal mucin-2 (MUC-2), zona occludens-1 (ZO-1), occludin (OCLN), and claudin-1 (CLDN-1) (R^2=0.24 to 0.46; $P<0.05$), and ileal MUC-2 and OCLN (R^2=0.26 to 0.57; $P<0.05$). Supplementing probiotics in the non-pathogen group positively correlated with the villus height (VH) and VH/ crypt depth (CD) ratio at week 3 or 6 of life both in jejunum (R^2=0.28 to 0.66; $P<0.05$) and ileum (R^2=0.41 to 0.58; $P<0.05$). Dietary probiotics in the non-pathogenic groups showed a positive relationship with daily weight gain (ADG; R^2=0.40 to 0.57; $P<0.05$) and a negative relationship with the feed conversion ratio (FCR; R^2 = 0.33 to 0.62; $P<0.001$) in starter, finisher, or whole periods. In the pathogenic groups, there were positive correlations between dietary probiotics and fold-change expression of ZO-1, OCLN, and CLDN-3 in the jejunum from week 2 to 4 of life (R^2=0.28 to 0.97; $P<0.05$), as well as of ZO-1 and OCLN in the ileum at week 4 (R^2=0.56 to 0.71; $P<0.05$). Moreover, negative relationships were identified between probiotics and fold-change values of the immune-related genes coding for interleukin-6 (IL-6), -10 (IL-10), and-1β (IL-1β), interferon-gamma (IFN-γ), and tumor necrosis factor-alpha from week 2 to 4 in the jejunum (R^2=0.35 to 0.99; $P<0.05$), as well as toll-like receptor-4 and IFN-γ at week 2 or 3 in the ileum (R^2=0.71 to 0.75; $P<0.05$), and IL-6 and -10 at week 2 in the ceca (R^2=0.31 to 0.47; $P<0.05$). Under pathogen challenge, probiotics positively correlated with VH and VH/CD ratio, but had a negative relationship with CD in the duodenum at week 5 (R^2=0.53; $P<0.05$), jejunum from week 2 to 5 (R^2=0.28 to 0.71; $P<0.05$), and ileum at week 5 (R^2=0.37 to 0.41; $P<0.05$). A positive and negative correlation was found between probiotics and ADG (R^2 = 0.25 to 0.67; $P<0.05$) and FCR (R^2 = 0.35 to 0.85; $P<0.05$) in the starter and overall period, respectively, and a positive relationship with ADFI in the finisher period (R^2 = 0.34; $P<0.05$).

Conclusions: Regression results showed that correlation between dietary probiotics and barrier and immune-related gene expression was greater in jejunum than in ileum or ceca either with or without pathogen challenge. Probiotics also improved the histomorphological measures in the small intestine which is crucial for nutrient uptake, thereby supporting performance in broilers challenged or not with pathogens.

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77.

Influence of feed additives and host-related factors on intestinal T cell distribution in broilers

Einfluss von Futterzusatzstoffen und wirtsspezifischen Faktoren auf die intestinale T-Zell-Verteilung in Broilern

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Intestinal epithelial lymphocytes are lymphoid cells that reside in the epithelium and the subepithelial space which participate in promoting barrier function as well as regulating mucosal immune responses. The mucosa-associated immune system may be influenced by age, genetic background, and dietary factors. This study investigated the effect of age, breed, sex and probiotic or phytobiotic supplementation as well as their interactions on $CD3^+$ T cell distribution in the ileum and caecum of broilers.

Methods: A total of 2,880 one-day-old male and female broiler chicks from two breeds, Ross-308 and Cobb-500, were randomly allocated to 72 pens. Each pen consisted of 40 chicks from each sex and each breed. Broilers were offered 3 experimental diets including a standard wheat-soybean based diet without or with supplementation of either a probiotic ($2.4x\ 10^9$ CFU/kg diet; Bacillus subtilis DSM32324 and DSM32325 and Bacillus amyloliquefaciens DSM25840) or a phytobiotic (165 ppm procyanidins and 585 ppm polyphenols) product. At day 7, 21, and 35 of age, one chicken per pen was sacrificed to dissect the middle part of the ileum and the distal part of the caecum. The intestinal tissue was stained with an anti-CD3 antibody. The positive cells ($CD3^+$) were counted in an area of 10,000 µm2 of the lamina epithelialis (LE) and lamina propria (LP) of the ileal villus (upper and lower part) and ileal crypt as well as the caecal crypt. Data were subjected to ANOVA using GLM procedure with a 3 (age) × 2 (breed) × 2 (sex) × 3 (diet) factorial arrangement of the main factors.

Results: The average $CD3^+$ density in the ileum was 27.4 + 0.97, 35.8 + 1.05 and 23.0 + 0.82 cells per 10,000 μm^2 of villus tip, villus base and crypt, respectively. The average caecal $CD3^+$ density was 20.6 + 0.68 cells per 10,000 μm^2. The horizontal distribution (LE and LP) of $CD3^+$ T cells in the ileum and caecum was varied; the ileal villus showed a greater $CD3^+$ density in the LE, while the ileal crypt and caecal crypt had a higher density in the LP ($p < 0.05$). The impact of the investigated main factors demonstrated that age affected $CD3^+$ density in the ileum and caecum ($p < 0.05$), but no impacts of breed, sex and diet were observed in both intestinal locations ($p > 0.05$). In the ileal villus, the horizontal distribution of $CD3^+$ T cells showed an increased density from day 7 to 21, with an increase by 125 and 96 % in the LE and LP at the villus tip, and by 105 and 65% in the respective sites at the villus base ($p < 0.05$). From day 21 to 35, the $CD3^+$ density was decreased by 23 and 41 % in the LE and LP at the villus tip and by 32 and 47 %, respectively, at the villus base ($p < 0.05$). In contrast, in the ileal crypt, the $CD3^+$ density increased with age, with approximately 87 and 185 % increase in the LE and LP, respectively, throughout the study period ($p < 0.05$). In the caecum, the horizontal distribution of $CD3^+$ T cells was increased with age, with approximately 111 and 76 % in the LE and LP, respectively, throughout the study period ($p < 0.05$). There was no interaction effect between the main factors was observed ($p > 0.05$).

Conclusions: The distribution of $CD3^+$ T cells in the ileum and caecum of broilers was substantially influenced by age. The $CD3^+$ density in the villus of ileum was highest on the third week of age, while the $CD3^+$ density in the ileal crypt and caecal crypt was increased until the fifth week. The fluctuation of $CD3^+$ density in the villus may be influenced by the contact of intestinal epithelium with luminal stimuli (e.g. gut microorganisms). The impact of breed and sex of broilers and the dietary treatments was not found on $CD3^+$ density in the ileum and caecum.

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78.

Effects of dietary grape extract supplementation on gut histomorphology and antioxidant parameters in small intestine and liver of piglets four and eight weeks post weaning

Effekte eines Traubenextraktes auf Darmhistomorphologie und antioxidative Parameter in Dünndarm und Leber von Ferkeln vier und acht Wochen nach dem Absetzen

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Grapes are rich in secondary plant constituents such as polyphenols, which are known for their antimicrobial and antioxidant properties in the gut after oral supplementation in monogastric animals. Especially in critical developing stages such as weaning, these properties of polyphenols might be favourable for gut maturation [1], thus reducing the prevalence of infections and the necessity of routine (pro- and metaphylactic) antibiotic treatments [2]. However, thorough evaluation of potential positive effects of secondary plant constituents in comparison to routine antibiotics as positive control is missing. We previously reported improvements of nutrient digestibility by grape extract (GpE) compared to routine antibiotics [3]. Thus, to deeper explore the mode of action of GpE, we evaluated morphological measurements in jejunum, ileum and colon, as well as antioxidant status in jejunum, ileum and liver tissue compared to negative and positive control.

Methods: At weaning, 180 piglets (6.9±0.1 kg body weight (BW)) were allocated to 3 treatment groups (6 male and 6 female pens each, 5 piglets/pen). Piglets received a corn-based diet without supplementation (negative control; NC), in-feed antibiotic in a therapeutic dosage (PC, 20 mg amoxicillin/kg BW every 12h; Amoxicillin-Trihydrate 100, 1000 mg/g) from day 1 to day 5 post weaning or GpE (150 g/t, dried extract from dried grapes (Vitis vinifera); ~ 40% total polyphenol content) diet supplementation for the whole duration. Diets were provided ad libitum as starter (d1-d13; 14.3 MJ/kg, 192 g CP/kg) and grower (d14-d56; 14.0 MJ/kg, 176 g CP/kg) diet. Upon euthanasia, tissue samples of intestine and liver were collected from 1 piglet/pen after 4 and 8 weeks of the trial. Statistical analyses were performed using MIXED procedure of SAS 9.4. Statistical model included treatment, sex, sampling day and the interactions of these factors. Least square means were compared by Tukey–Kramer test and differences were considered significant at $p < 0.05$.

Results: After 4 weeks of the trial, villus surface in jejunum was increased by GpE and PC, and GpE increased villus height compared to both, NC and PC ($p<0.05$). In ileum, an increase of villus height, surface area, VC-Ratio as well as the number of villus goblet cells was observed after 8 weeks, in GpE and PC group ($p<0.05$). There were no significant effects of the dietary treatments on antioxidant enzyme (glutathione peroxidase, catalase, superoxide dismutase) activity, level of lipid peroxidation (thiobarbituric acid reactive substances, TBARS) or antioxidant capacity measured by 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2‘-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) methods in intestine and liver, for both time points analysed post weaning.

Conclusions: The GpE inclusion showed benefits for the growth of small intestinal villi, however, we observed no improvement of oxidant stability compared to NC and PC. Obviously, changes in villus architecture may be connected with the improvement of nutrient digestibility [3]. However, antioxidant properties of GpE – in the chosen concentration and duration of supplementation, sampling time points and selected measurements – were not the mechanism behind the improvement.

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79.

Effects of dried alfalfa or timothy as chaff or pellets fed in different proportions to oat grains in a hay-based diet vs hay plus oats and hay alone on the gastric mucosa of horses

Einfluss von Luzerne- oder Wiesenlieschgras-Trockengrün als Häcksel oder Pellets in unterschiedlichen Gemengeanteilen zu Hafer in einer heubasierten Ration im Vergleich zu Heu und Hafer bzw. nur Heu auf die Magenschleimhaut von Pferden

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Alfalfa (ALF) chaff (CH) is very popular as structural component in crib feed of horses. However, previous studies with weaning foals [1] and adult horses [2] indicate that ALF-CH might pose a risk for gastric health, at least when high amounts are fed. This is probably because the hard stems induce traumatic mucosal lesions in the antrum. Nevertheless, it is questionable which effect common proportions of ALF-CH or CH of another botanical origin (grass, GRA) or pellets from ALF and GRA have in this context, respectively. The objective of the current study was to compare effects of dried ALF or GRA (here: timothy) as CH or PE fed in different proportions to whole oat grains (OG) in a meadow hay-based diet vs hay plus OG and hay alone on the gastric mucosa of horses. Inclusion rates of ALF and GRA products in the crib feed were planned to vary between what is common in practice (10%) and very high (50%).

Methods: In a cross over design, 6 warmblood mares (age 15 ± 1.1 years; body weight [bwt] 553 ± 39.0 kg; body condition score 5.6 ± 0.19/9) were allocated to 14 diets with either hay alone or hay (1.5 kg/100 kg bwt and day) plus OG or crib feed consisting of OG mixed with 10, 30 and 50% (by weight as fed) of ALF or GRA, either as CH or PE (Ø 5 mm). Both products were made from uniform batches of ALF or GRA. Energy and nutrient contents of the feedstuffs were as follows (per kg dry matter [dm]): GRA-CH, 89 g crude protein (CP), 12 g crude lipids (CL), 398 g acid detergent fiber (ADFom), 6.3 MJ metabolizable energy (ME); GRA-PE, 118 g CP, 16 g CL, 376 g ADFom, 6.1 MJ ME; ALF-CH: 146 g CP, 18 g CL, 411 g ADFom, 5.4 MJ ME; ALF-PE, 180 g CP, 20 g CL, 321 g ADFom, 6.3 MJ ME; OG, 110 g CP, 55 g CL, 154 g ADFom, 446 g starch, 12.6 MJ ME. Hay and crib feed were provided in two equal meals per day. Straw from the bedding, salt lick and tap water were freely available. The horses had light training on a treadmill for 5 days a week and daily paddock turnout. The diets were composed to cover the energy need of around 1.1-fold maintenance level. Each feeding period lasted 4 weeks and gastroscopic examination was performed at the end of the last week. Gastric mucosa of the Pars nonglandularis (PN) and Pars glandularis (PG) were scored according to what is summarized in [3] and graded from 0 (intact epithelium) to 4 (extensive/deep lesions) to objectify as much as possible Equine squamous gastric disease (ESGD) and Equine glandular gastric diesease (EGGD), respectively Scores were expressed as LSMeans ± pooled se and effects (fixed: plant type, treatment, inclusion rate; random: horse) analyzed using the procedure GLIMMIX (SAS 9.4).

Results: With hay only and hay plus OG, ESGD scores were 0.76 and 1.11, and EGGD scores 1.94 and 1.36. GRA-CH, ALF-CH, GRA-PE and ALF-PE at different inclusion rates caused ESGD scores of 0.38 – 1.27, 0.55 – 0.90, 0.66 – 0.82 and 0.06 – 0.79 (± 0.42), and EGGD scores of 1.59 – 1.91, 1.14 – 1.56, 1.51 – 2.31 and 1.50 – 1.91 (± 0.47). Both mucosal regions rather tended to respond differently to feeding of the various diets. Despite highest scores occurred predominantly at 30% and 50% inclusion rates, there was neither a rectilinear impact of the proportion of ALF and GRA products in the crib feed nor was there a clear diet effect on either score ($P > 0.05$). Animal-individual differences seemed to overlay any diet effect.

Conclusions: The inclusion of CH or PE from ALF or GRA up to 50% to oat grains seems not to cause detectable alterations of the ESGD and EGGD score, respectively, at least if diets were fed that largely correspond to species-specific requirements with high amounts of roughage and starch intake < 1 g/kg bwt and meal. Surprisingly, hay alone seems not to be superior protective on gastric mucosa under these conditions.

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80.

A large-scale farm study reveals links between the nutritional status with fertility and udder health of dairy cows in Austria

Eine großangelegte Praxisstudie zeigt einen Zusammenhang des Ernährungszustandes mit der Fruchtbarkeit und Eutergesundheit von Milchkühen in Österreich

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In practice, the influence of nutrition on udder health and fertility performance is often overlooked since feeding is mainly viewed to affect the performance of dairy cows. Indeed, imbalances of nutrients especially energy and protein could offset metabolic homeostasis and consequently affect health and reproductive performance. In general, less is known about current situations regarding the feeding and nutritional status of dairy cows in commercial farms. This study identified differences in nutritional status, fertility and udder health traits as well as the risk factors for decreased fertility performance of cows in Austrian dairy farms.

Methods: Following the farmers' consent, 100 dairy farms located in Upper Austria (n = 51), Lower Austria (n = 33) and Styria (n = 16) were included in a two-year study. Farm interviews and feed sampling were performed twice per farm during 2019 – 2020 to obtain data for the nutrient composition, estimated intake, particle distribution, and main ingredients (total n = 198). The annual fertility and milk data were obtained from the national reporting agency (Zuchtdata EDV-Dienstleistungen GmbH, Austria). Calving interval and the proportion of primiparous or multiparous cows with high somatic cell counts (SCC) >200,000 cells/ml of milk were the targeted fertility and udder health, respectively. Each of these variables was categorized into 3 groups: low, high and norm following the 25th and 75th percentile and the rest of the data, respectively. The category was tested as the fixed effect in a mixed model in which the repeated visits were considered as the random factor. In addition, we determined the links between herds with apparent udder issues (according to the SCC) or metabolism issues (according to milk component variables) with the length of the calving interval. For this, comparisons among the calving interval categories using the mixed model as well as fitting a linear logistic regression model using the logistic procedure of SAS comparing the long and short calving interval data were performed.

Results: Simmental was the main breed in the herd (84% ± 30, mean ± SD). The content of non-fiber carbohydrates (NFC) in the basal diet was higher in the group with long calving intervals (≥ 400 d, n = 44) and the group with high SCC incident in primiparous cows (≥ 20% of the herd, n = 38) compared to their low counterpart (≤ 380 d, n = 62 and ≤ 8% of the herd, n = 46, respectively) ($P < 0.05$). The high SCC incident group also showed a higher level of maize silage in the diet compared with the low incident group (26.9 vs. 22.9% of diet DM, $P < 0.05$). Linear regression analyses indicated a positive relationship between the NFC content and calving interval: $Y = 376.4$ (SE = ±5.3, $P < 0.001$) + 0.576 (±0.21, $P < 0.01$) × NFC (% of basal diet DM), and the maize silage content and the percentage of primiparous cows with SCC > 200,000 cells/ml: $Y = 11.05$ (±1.05, $P < 0.001$) + 0.141 (±0.036, $P < 0.01$) × maize silage (% of diet DM). Herds with high proportions of cows with high milk fat to protein ratios (> 1.5) had an increased risk for long calving intervals. This was found for both primiparous (odds ratio = 5.5, 95% confidence interval (CI) = 1.65 - 21.7, $P = 0.009$) and multiparous cows (odds ratio = 4.08, CI = 0.98 - 18.62, $P = 0.058$). Herds with multiparous cows with high milk urea nitrogen (>30 mg/dl) also increased the risk for longer calving interval (odds ratio = 2.96, CI = 1.22 - 7.87, $P = 0.021$). The long calving interval group had a higher percentage of primiparous ($P < 0.05$) and multiparous cows ($P < 0.01$) with SCC > 200,000 cells/ml compared to the short calving interval group. However, they were insignificant based on the logistic regression model.

Conclusions: The present work underlines the prevalence of farms with substandard nutritional status related to the energy and protein of cows in lactation that may be linked to decreased fertility performance.

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81.

A systematic review of worldwide calf management on dairy farms focusing on calf feeding practices

Systematische Literaturanalyse des weltweiten Kälbermanagements auf Milchviehbetrieben mit besonderem Fokus auf Fütterungspraktiken

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In recent decades, there have been many significant changes in the international dairy industry, leading to growing public concerns about dairy calf management practices. Empirical studies about dairy calf management practices conducted worldwide were designed to identify areas where scientific recommendations to improve calves' health, welfare, and development are well or poorly translated into practice. A systematic review of the published data would provide valuable insights into the differences between countries and common challenges in implicating calf management recommendations to improve knowledge transfer. Hence, this systematic review aimed to analyze published empirical studies on dairy calf management regarding the implementation of recommendations into practice worldwide.

Methods: The systematic review was performed following Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines. Relevant survey studies were found through targeted searches in the Web of Science database using defined combinations of keywords and the reference lists of articles found. Used terms were variating versions of "calves" and "dairy calves" combined with the Boolean operator "AND" with variations of the terms "management," "practices," or "survey." Publications were included if they were published in a peer-reviewed journal in English between 2010 and 2021, the full text was accessible, and they covered at least two topics of calf management (e.g., colostrum management and feeding management). From an initial pool of 576 publications, 33 articles covering different breeds were included, systematically analyzed, and compared by continent and country.

Results: Most included empirical studies were conducted in Europe (n = 12), followed by North America, South America, and Asia (n = 5, each) and Africa and Australia (n = 3, for both). Early separation of calf and dam after birth (<12 h p.p.) seems common on European, North American, and Brazilian farms. In contrast, in Chile, Uruguay, Australia, and Bangladesh, separation is most often performed after 24 h or later. Colostrum quality is only rarely checked in every country (range: 0–51 % of farms surveyed checked quality). Most European and North American studies reported that farmers feed colostrum manually in quantities between 2-4 L, whereas in South American countries, calves dominantly receive colostrum by suckling the dam. Ad libitum milk feeding is uncommon in all countries included (maximum of 12 % of farmers in an Austrian study), and feeding restrictively, low quantities (< 6 L/day) of milk is most common on all continents and most countries. Feeding of waste milk has been reported in twelve studies from ten countries and seems to be common (range: 34% of farms in the Czech Republic to 84% in Austria). Water and solid feed are provided in most countries and farms within the first two weeks. However, farms in Uruguay, Pakistan, India, and Senegal differed as some stated not to provide concentrate preweaning at all.

Conclusions: This review indicated that calf feeding management on farms differed in some points between countries (e.g., colostrum feeding or separation of dam and calf). However, some recommendations seem to be rarely implemented in nearly all countries included (e.g., checking colostrum, avoiding waste milk feeding, and feeding high quantities of milk). Further analyzing the identified countries in which recommendations were implemented could give valuable insights into effective knowledge transfer. Furthermore, recommendations that were poorly implemented in all countries hint at limitations of the already existing measures in these countries.

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82.

Effects of dried alfalfa or timothy chaff or pellets fed in different proportions to oat grains on chewing patterns of horses

Einfluss von Luzerne- oder Wiesenlieschgras-Trockengrün als Häcksel oder Pellets in unterschiedlichen Gemengeanteilen mit Hafer auf Kauparameter von Pferden

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Chaff is very popular as a structural component in the crib feed of horses. However, it is questionable whether alfalfa and grass chaff stimulate chewing to the same extent and in what quantities they are needed for this purpose. Data comparing chaff and pellets from the same batches of grass or alfalfa are also missing. The aim was to compare effects of dried alfalfa or grass as chaff or pellets fed in different proportions to oat grains in a hay-based diet vs hay plus oat grains on chewing patterns of horses. The proportion of alfalfa and grass products in the crib feed was planned to vary from more common (10%) to very high (50%).

Methods: 6 warmblood mares (age 15 ± 1.1 years; body weight [bwt] 553 ± 39.0 kg; body condition score 5.6 ± 0.19/9) were allocated to 14 diets with either meadow hay alone or hay (1.5 kg/100 kg bwt and d) plus whole oat grains mixed with 0, 10, 30 or 50% (by weight as fed) of alfalfa or grass (here: timothy) chaff or pellets (Ø 5 mm). Each horse received each diet for 4 weeks with season effects were factored out by a cross over design. Chaff and pellets were manufactured from uniform batches. Energy and nutrient contents were as follows (per kg dry matter [dm]): grass chaff, 89 g crude protein (CP), 12 g crude lipids (CL), 398 g acid detergent fiber (ADFom), 6.3 MJ metabolizable energy (ME); grass pellets, 118 g CP, 16 g CL, 376 g ADFom, 6.1 MJ ME; alfalfa chaff: 146 g CP, 18 g CL, 411 g ADFom, 5.4 MJ ME; alfalfa pellets, 180 g CP, 20 g CL, 321 g ADFom, 6.3 MJ ME; OG, 110 g CP, 55 g CL, 154 g ADFom, 446 g starch, 12.6 MJ ME. Hay and subsequently crib feed were fed in two equal meals per day. Straw from the bedding, salt lick and tap water were freely available. The horses had light training and diets covered ≈ 1.1-fold maintenance energy need [1]. In the last week of each feeding period, at both mealtimes, 2 kg of hay were fed 1 h prior to the crib feed. Feed intake time (FIT, in min/kg dm) and count of chews (CC) were measured, the latter 10 min after crib feed supply, by modified halters [2] and analyzed for chewing frequency and chewing intensity. Scores were expressed as LSMeans ± pooled se and effects (fixed: plant type, treatment, inclusion rate; random: horse) analyzed using the procedure GLIMMIX (SAS 9.4).

Results: Adding chaff to oat grains (oat grain only: meal 1, FIT 10.7 ± 2.02 min/kg dm, CI 784 ± 129.8 CC/kg dm; meal 2, FIT 11,0 ± 1.92 min/kg dm, CI 822 ± 129.3 CC/kg dm) increased FIT and CI from a proportion of 30% for grass and 50% for alfalfa ($P < 0.05$). 50% grass pellets elevated CI at meal 1 ($P < 0.05$). The CF ranged from 1.15 – 1.50 CC/s and was not affected by the diet ($P > 0.05$).

Conclusions: Advantageously, alfalfa and grass chaff to oat grains increase FIT and CI, but rather high proportions (here: 30% for grass chaff, 50% for alfalfa chaff) are needed for this. Interestingly, high proportions of grass pellets (here: 50%) also seem to elevate CI. This contradicts previous results with pelleted compound feed which had the same diameter but was fed as the only crib feed [2]. Future research might show whether this contradiction bases on pellet characteristics (composition, hardness etc.) or the supply in a mix instead of alone.

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83.

Incidence of ESBL-*E. coli* in nipples of teat buckets for calves before and after automatic cleaning in two dairies

Vorkommen von ESBL-E. coli in Nuckeln von Kälbertränkeeimern vor und nach der maschinellen Reinigung in zwei Milchviehbetrieben

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Young calves often carry extended spectrum beta-lactamase producing E. *coli* (ESBL-*E. coli*). Feeding of waste milk containing antibiotic residues could be identified as a risk factor for high ESBL-*E. coli* load in calves [1]. However, also the direct transmission of ESBL-*E. coli* via the colostrum meal into the faecal microbiome of calves could be demonstrated [2]. Usually, ESBL-*E. coli* did not occur in the healthy udder, but rather enter colostrum or milk through the equipment for harvesting and feeding [2,3]. Therefore, the aim of the present study was to investigate if ESBL-*E. coli* can routinely be detected in the inner surface of nipples of teat buckets for calves and if an automatic wash system developed for teat buckets is suitable to eliminate existing ESBL-*E. coli*.

Methods: Overall, 120 swaps (Amies) were taken; actually the faeces of 40 calves from two different herds and the inner surface of the nipples of the teat buckets they used before and after washing procedure were sampled. Detection of ESBL-*E. coli* was done by Cefotaxim-containing CHROMagar. The incidence of ESBL-*E. coli* before and after automatic cleaning with the automatic wash system for teat buckets (GRETE GmbH) was statistically analyzed via chi-square test. To evaluate the differences in teat bucket hygiene in the two farms, several parameters were recorded, e. g. waste milk feeding, detergents used in the automatic wash system, additional manual hygiene measures, change of nipples etc.

Results: All sampled calves were tested ESBL-*E. coli* positive which might be due to waste milk feeding in both herds. In farm 1, even all 20 nipples carried ESBL-*E. coli* in the inner surface before purification. In farm 2, only in one of 20 nipples ESBL-*E. coli* could be detected. After cleaning the nipples of farm 2 were negative, whereas in farm 1 the automatic washing procedure did not eliminate all ESBL-*E. coli*; 6 nipples remained ESBL-*E. coli* positive. Statistically the ESBL-*E. coli* load was significantly reduced through automatic cleaning with the GRETE system ($P < 0.0001$). A potential explanation for the differences in the incidences of ESBL-*E. coli* in the inner surface of the nipples of the two dairies are maybe the differences in teat bucket hygiene: In contrast to farm 1 , farm 2 had more buckets than calves, therefore, in daily farm routine all buckets could be cleaned with the automatic wash system and dried on a special rack before the next feeding time. Furthermore, in farm 2 the buckets were additionally cleaned by hand every five weeks and the nipples of the buckets were routinely substituted every 21 days, whereas, in farm 1 the nipples were only replaced when they were damaged.

Conclusions: Cleaning in an automatic wash system for teat buckets reduces the ESBL-*E. coli* load of the inner surface of nipples of teat buckets for calves. However, it seems that further measures are necessary to exclude the occurrence of ESBL-*E. coli* in nipples of teat buckets to prevent the transmission of ESBL-*E. coli* via the nipples. Moreover, known risk factors for the occurrence of ESBL-*E. coli*, e. g. waste milk feeding, should be avoided in dairy farms to reduce the ESBL-*E. coli* prevalence in calves.

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84.

Plasma metabolomics profiling of nematode infected chickens

Plasma-Metabolom von mit Nematoden infizierten Hühnern

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Ascarid infections reduce laying performance in chickens through impaired absorption and utilisation of nutrients. High performing genotype chickens are particularly sensitive to these adverse effects [1]. Therefore, this study was conducted to identify key alterations in the plasma metabolome of high performing hens of Lohman Brown (LB) genotype with or without exposure to *Ascaridia galli* and *Heterakis gallinarum* infections.

Methods: A total of 108 hens of the LB genotype at the start of laying period (i.e., 24-week-old) were either orally infected with 1000 embryonated eggs of both *A. galli* and *H. gallinarum* or kept as control. Plasma samples were collected from hens at slaughter at 2, 4, 6, 10, 14 and 18 week-post infection (wpi). Metabolomics profiles of the plasma were assessed using NMR spectroscopy. ^{1}H-NMR spectra were acquired at 310 K on a 14 T Bruker Avance III spectrometer (Bruker BioSpin, Rheinstetten, Germany) equipped with a 5 mm TXI probe head with gradients, automated tuning, and matching accessory (ATMATM), BCU-I for regulation of temperature, and SampleJet robot cooling system set to 5 °C as a sample exchanger. For quantification, metabolite peaks were integrated and quantified relative to the added internal standard Trimethylsilylpropanoic acid (TMSP-d4) using the Chenomix software. Univariate analyses (Volcano plot and significant analysis of microarrays) were performed to identify metabolites that differ significantly between infection and control groups, and a pathway enrichment analysis was conducted to identify significant pathways associated with response to the mixed nematode infections using the MetaboAnalyst software (MetaboAnalyst 5.0).

Results: From the obtained ^{1}H-NMR spectra compared with the reference data from Chenomix software, a total of 31 metabolites were identified within both treatment groups across all wpi. Among them, amino acids and analogues, and organic acids were the dominant metabolite classes identified. Concentrations of 23 metabolites were significantly higher in infected hens than in non-infected hens ($P<0.05$). The changes in the metabolites' concentrations were also time-dependent with most significant alterations occurring at wpi 2, 6, and 10, implying early-phase infections and patency are associated with metabolic changes. Key changes included the metabolites glutamate, succinate, trimethylamine-N-oxide, creatine, and alanine. Enrichment analysis showed that infection-induced upregulation was strongest in arginine and proline, and D-glutamine metabolic pathways.

Conclusions: In conclusion, the plasma metabolome of LB chickens infected with *A. galli* and *H. gallinarum* revealed alterations that were dependent on both the presence and the patency of infections. Infection tended to upregulate key metabolic pathways.

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85.

Influence of Cryptosporidium parvum infection on glucose uptake and metabolism in neonatal calves

Einfluss einer Infektion mit Cryptosporidium parvum auf den Glukosetransport und -stoffwechsel in neonatalen Kälbern

*Dengler F., Hammon H. M., Liermann W., Görs S., Bachmann L., Helm C., Ulrich R., Delling C. – Vienna / Dummerstorf / Neubrandenburg / Leipzig

Cryptosporidiosis is one of the main causes of diarrhea in neonatal calves, posing an immense economic problem in calf rearing and negatively affecting animal welfare. So far, there is no fully effective therapy available, which is at least partly due to lacking understanding of the pathophysiology. Previous studies in cell culture models indicated an interference with intestinal glucose uptake and metabolism which may be caused by the nutritional demand of the parasite [1]. Therefore, this pilot project aimed to investigate the effects of infection with C. parvum in neonatal calves on the transepithelial transport and systemic metabolism of glucose *in vivo*.

Methods: Neonatal calves were infected with $2x10^7$C. parvum oocysts (INF, N=5) on the first day of life, while a control group (CON, N=3-5) was administered H_2O orally. The feeding regime was identical in all animals, consisting of 3L pooled high quality colostrum after birth followed by 3 x 2L milk replacer daily. Clinical parameters and faecal shedding of C. parvum oocysts were monitored for one week. 6 days post infection (p.i.) glucose absorption, turnover and oxidation were assessed using stable isotope labelled glucose ($[^2H_2]$-glucose intravenously and $[^{13}C_6]$-glucose per os). On day 7 p.i., calves were sacrificed, isolated jejunum epithelium was mounted in Ussing chambers and the electrogenic glucose uptake via Na^+-linked transport (SGLT1) was evaluated. Glucose transporters were quantified on gene and protein expression level using RT-qPCR and Western blot in the jejunum epithelium and brush border membrane preparations. The gene expression of enzymes involved in glucose metabolism was assessed by RT-qPCR.

Results: Plasma glucose concentrations of INF were lower compared to CON on day 6 and 7 p.i. ($p<0.05$; two-way RM ANOVA + Holm-Sidak-test). In line with this, the appearance rate of orally applied glucose was significantly higher in CON compared to INF ($p=0.02$; t-test), whereas the appearance rate of $^{13}CO_2$, a proxy for the oxidation of oral glucose, was significantly higher in INF compared to CON ($p=0.026$; t-test). The glucose turnover was similar in both groups. Ussing chamber experiments revealed a significant increase of phlorizin-sensitive electrogenic transport of glucose across the infected epithelia compared to CON ($p<0.05$; two-way RM ANOVA + Holm-Sidak-test). The tissue conductance was similar in all groups, indicating a sustained barrier function. There was no difference in the gene or protein expression of glucose transporters, but an enrichment of glucose transporter 2 in the brush border was observed in the infected calves ($p=0.059$; t-test). Furthermore, the mRNA expression of hexokinase 2 and phosphofructokinase was significantly increased in INF compared to CON ($p<0.05$; t-test).

Conclusions: Our results indicate an upregulation of the glucose uptake and metabolism machinery in *C. parvum* infected jejunum epithelium at simultaneously lower systemic blood glucose levels and reduced oral glucose appearance rate in infected animals. This might indicate an increased metabolic demand due to the infection and/or a nutritional competition of the parasite, diminishing the glucose supply of the host. In contrast to the prevailing view that glucose uptake is disturbed in *C. parvum* infection, the enterocytes seem to attempt to compensate and increase the transport capacities for glucose uptake.

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86.

Effects of abomasal fatty acid supplementation on the intestinal fatty acid composition and barrier function in lactating cows

Einfluss abomasaler Fettsäuresupplementation auf die intestinale Fettsäurezusamensetzung und Barrierefunktion in laktierenden Kühen

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Polyunsaturated fatty acids (PUFA) play an important role in the maintenance of the intestinal health [1], but their effects on intestinal permeability were controversially discussed. Thus, in general n-3 PUFA are able to support intestinal barrier function; however, eicosapentaenoic acid (EPA) or docosahexaenoic acid (DHA) leading under some circumstances to hyperpermeability [2, 3]. A current study demonstrated an beneficial impact of conjugated linoleic acids on the intestinal permeability in mice (3). The present study aimed to investigate the influences of essential fatty acids and conjugated linoleic acids on the intestinal fatty acid composition, mucin and tight junction expression in the intestine of lactating cows.

Methods: A total of 38 fistulated Holstein cows were supplemented abomasally either with 76 g/d coconut oil (CON, n = 9), essential fatty acids (EFA, n = 9; 74 g/d linseed oil and 4 g/d safflower oil), conjugated linoleic acid (CLA, n = 10; 38 g/d Lutalin®) or with a combination of EFA and CLA (EFA+CLA, n = 10; 38 g/d Lutalin®, 78 g/d linseed oil and 4 g/d safflower oil) from 9 weeks before until 9 weeks after calving. During the dry period, doses were halved. Cows were slaughtered 9 weeks after calving and jejunal samples were collected. The study was divided into five blocks. The proportions of specific fatty acids out of the total amount of fatty acids in jejunum were determined by gas chromatography. The gene expression of *Zonula Occludens 1* (ZO-1), occludin (OCLN), claudins (CLDN 1, 4) and mucin-2 (MUC2) were analysed by quantitative real time PCR. ZO-1 protein expression was determined by ELISA. For statistical analyses the MIXED procedure of SAS was used including the fixed effects CLA and EFA as well as their interactions. The block was also defined as a fixed factor and the breeding interval as well as the milk production of the preceding lactation period were considered as co-variables.

Results: Conjugated linoleic acid supplementation increased CLA *cis*-9, *trans*-11, linoleic acid, sum of n-3, n-6 and PUFA in the jejunum but decreased EPA and the sum of monounsaturated and saturated fatty acids ($P < 0.05$). EFA supplementation increased proportions of α-linolenic acid, EPA and the sum of n-3 and PUFA but decreased the proportions of the sum of n-6 fatty acids and monounsaturated fatty acids. No interactions between EFA and CLA were observed in case of the fatty acid composition in the intestine ($P > 0.05$). MUC2 gene expression tended to increase by CLA compared to CON ($P < 0.1$). There was no difference in the gene expression of CLDN1, CLDN4 and OCLN between the groups. However, ZO-1 gene expression tended to be higher in EFA supplemented cows compared to cows with no EFA supply and ZO-1 protein expression tended to be higher in the CLA+EFA group compared to the cows only supplemented with CLA ($P < 0.1$).

Conclusions: Abomasal infusion of EFA and CLA affected the intestinal fatty acid composition of dairy cows. Elevated n-3 PUFA and CLA might contribute to modulations of the intestinal barrier function as indicated by the elevated gene expression of MUC2 and ZO-1 as well as a trend to increased protein expression of ZO-1.

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87.

Composition of the faecal microbiome of old vs younger adult horses under similar housing and feeding conditions

Zusammensetzung des fäkalen Mikrobioms alter vs. jüngerer Pferde unter vergleichbaren Haltungs- und Fütterungsbedingungen

*Pisch C., Rechenburg L., Glatter M., Wensch-Dorendorf M., Zeyner A. – Halle (Saale)

Even healthy old horses (age > 20 yrs.) show remarkable anatomical and physiological alterations which also include the digestive tract. Diverse faecal parameters comprising microbial fermentation products are suitable to characterize microbial activity and milieu conditions in the posterior part of the large intestine and partly change with age [1,2]. Previously it has been indicated that old vs young horses show lower faecal microbial diversity, but this study was conducted under experimental feeding conditions rather unusual for old horses [3]. The objective was to investigate whether the composition of the faecal microbiome differs with age in healthy old (OH) vs younger horses (YH) under similar housing and feeding conditions during winter and summer season when fed as common in practice.

Methods: From a riding stable, 14 old (21 - 31 years; 4 mares, 10 geldings; age 25 ± 3.4) and 14 younger (6 - 17 years; 6 mares, 8 geldings; age 11 ± 3.0) warmblood type horses were subjected to the study. The horses had no health problems that would have restricted mobility or received medication for the last four weeks prior to the study. All equines were dewormed and vaccinated regularly, had 6 hours per day paddock (winter: February) or pasture (summer: June) turnout and additionally moved for leisure. The stalls were equipped with wheat straw as bedding (2 horses also wood shavings), automatic waterers and a salt lick. Three times a day, 3 kg of meadow hay and 0.5 - 1.5 kg of concentrate (mostly oats:barley, 1:1) were fed. Two old horses were additionally fed a small amount of hay cobs. Fresh morning faecal samples were collected on the test day in both seasons and stored for later detection of dry matter, short chain fatty acids, ammonia and L- / D-lactate in the faecal innate water [1]. Faeces samples were used for sequencing of 16S rRNA amplicons performed on the Illumina HiSeq system (Illumina Inc., San Diego, CA, USA). For taxonomic assignments, the SILVA 132 rRNA database was used. The available number of sequences was normalized among samples by rarefaction. Two-way PERMANOVA and a principal coordinate analysis (PCoA) were performed in PAST version 4.01 based on Bray-Curtis similarity considering age and season (February or June). Shannon α-diversity, Simpson 1-D, Simpson evenness, and Menhinick species richness were calculated using QIIME 2. Kruskal-Wallis and Wilcoxon test were used for comparison of means in SAS version 9.4 (SAS Institute Inc., Cary, NC, USA) to identify differences in these metrics among age stages and between seasons, respectively. The significance level for all tests was pre-set at $P < 0.05$.

Results: At the genus level, age differences ($P < 0.048$) and a clear seasonal effect ($P < 0.001$) were detected for amplicon sequence variants. PCoA showed clustering of bacterial genera by season and trends ($P > 0.05$) related to the corresponding age classes. Shannon α-diversity and Simpson 1-D were not affected by age or season. Simpson evenness did not differ among age stages but was higher in the summer season ($P < 0.01$). Menhinick species richness was higher in younger (< 20 yrs.) than old horses (> 20 yrs. of age) ($P < 0.01$) and higher in summer than winter (February) ($P < 0.05$).

Conclusions: The results of the current study indicate a variable bacterial composition of horses once due to the age (here: > 25 vs< 20 years of age) but also because of the season and the associated different ration types (stable vs partial pasture feeding). Old vs younger horses show a reduced faecal microbial diversity also under practical feeding conditions which confirms the results of Dougal et al. [2] obtained under experimental conditions. A reduced faecal microbial diversity might indicate an elevated risk for digestive disorders located in the large intestine including impaction and probably the occurrence of faecal free liquids which partly age-related problems are well known from practice.

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88.

Investigation of the rumen digesta washing mechanism in live cattle

Untersuchung des Wasch-Effektes im Pansen von Kühen

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It has been suggested that particle sorting in the reticulorumen (RR) leads to a washing of the digesta before regurgitation and rumination [1]. This has been used to explain differences in dental anatomy and ingestive chewing between ruminants as compared to nonruminants. Yet, so far, this assumption has only been supported by results of slaughter experiments [2], where dorsal rumen contents, from which regurgitate for rumination is typically recruited, were comparatively depleted in abrasives. However, no studies in live animals with actual measurements of the regurgitate exist so far.

Methods: We tested the effect of contaminating a basal diet with sand in four rumen-fistulated Brown Swiss cows (approximately 5 years old, mean ± SD body mass 705 ± 64 kg). Two animals were in late lactation, receiving a diet of grass silage (chopped to a size of approximately 5 cm). The other two were in the galting phase and received a diet consisting of one-third of chopped straw (also at a particle size of approximately 5 cm) and two-thirds of the grass silage (mixed on an as fed-basis), to which water was added at twice the amount of the straw. Feed and water intake were measured daily. The diets were offered for ad libitum consumption for 29 days; only on day 18, there was a 6-hour period without access to feed (see below). From day 8 to 18, the basal diets were contaminated (by careful homogenous mixing) with sand at 2% as-fed (approximately 6 % dry matter, corresponding to levels reported in free-ranging herbivores). Diet, regurgitate (from the oral cavity) and faeces were sampled daily; leftovers were sampled from day 11 to 18; ingested feed boli were sampled via the fistula during eating every second day until day 17. On day 18, RR contents were sampled from the Atrium ruminis, dorsal, middle and ventral rumen contents at 0, 10, 20, 40, 60, 120 and 360 min after feeding. All samples were analyzed for acid detergent insoluble ash (ADIA) as a measure of silica. During a total of 8 days without and 8 days with sand contaminated feed, chewing behaviour was monitored using Rumiwatch chewing halters. Data analyses included paired Wilcoxon signed rank tests, linear mixed effects models and generalized additive models.

Results: There was no indication for discomfort or clinical problems during sand ingestion, for selective avoidance of sand, or for a reduction of feed intake during sand contamination. Water intake was not affected by sand contamination. Chewing behaviour did not change during the sand period. The ADIA concentration in the feed swallowed during ingestion (sampled via fistula) and, with two-day delay, in faeces, both reflected sand feeding. However, the ADIA concentration in the regurgitate remained close to baseline values. There was an immediate reduction in ADIA concentration from feed to rumen contents, and this reduction continued in dorsal rumen contents over time. With a slight intermittent increase in the middle layer of rumen contents, ADIA concentrations increased in the ventral rumen contents over time.

Conclusions: It has been claimed that cattle may not show adverse signs even when consuming, with their feed, 10 kg of sand per day for more than 30 days (3). The experiences during the present study, where the cows ingested about 0.6-1.1 kg of sand per day for 11 days in row, do not contradict that claim. Likely, soil ingestion is not uncommon for free-ranging herbivores, domestic ruminants at pasture, or domestic ruminants fed on silages, and need not automatically be considered a health threat. Our findings explain why ruminants are able to tolerate high levels of dust or grit in their diet with less high-crowned teeth than nonruminants in the same habitat. While the digesta washing need not be considered the major function for which the ruminant forestomach developed during evolution, it represents an inadvertent advantage that likely contributed to the ruminants' current success in terms of species diversity.

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89.

Transfer of pyrrolizidine alkaloids (PAs) into milk in a chronic PA exposure scenario of dairy cows

Transfer von Pyrrolizidinalkaloiden (PA) in die Milch in einem chronischen PA-Expositions-Szenario bei Milchkühen

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The increasing spread of ragworts (*Jacobaea spp., syn. Senecio*) might pose a risk for the health of grazing animals and humans consuming food deriving from those animals. Ragworts like tansy ragwort (*Jacobaea vulgaris Gaertn., syn. Senecio jacobaea L.*) or marsh ragwort (*Jacobaea aquatica*) contain hepatotoxic pyrrolizidine alkaloids (PAs) and their corresponding N-*oxides* (PANOs), hereinafter collectively referred to as PAs. Grazing animals usually avoid ragworts if pasture management is appropriate. However, preserved forage (silage, hay) originating from ragwort-contaminated pastures can lead to significant exposure to PAs giving rise to a possible transfer into food of animal origin, including milk. The aim of the present study was to investigate the transfer of PAs originating from a well-defined PA-extract into milk of dairy cows.

Methods: 20 German Holstein cows (body weight (BW) 649 ± 51 kg, 2nd lactation, days in lactation 170 ± 30) were assigned to five groups (n=4), whereof three groups received an increasing daily oral bolus of a well-characterised *Jacobaea vulgaris* extract for 28 days (PA1: 0.47 mg PA/kg BW/day (d); PA2: 0.95 mg PA/kg BW/d; PA3: 1.91 mg PA/kg BW/d). Two control groups were dosed either with water or molasses to account for the sugar content of the PA-extract. Feed (total mixed ration: 30% each grass and maize silage, 40% concentrate on dry matter (DM) basis) and water were offered ad libitum at self-feeding stations (Insentec, B.V., Marknesse, The Netherlands). Twice a day, the cows were milked. Milk yield as well as BW were recorded automatically (Lemmer Fullwood GmbH, Lohmar, Germany). The administration of the PA-boli into the reticulo-rumen was performed daily after the morning milking by using a stomach tube. LC-MS/MS analyses of the extract were carried out by two independent laboratories [1, 2], whereby the mean values of both results were used. Additionally, PAs in milk were analysed using solid-phase extraction and LC-MS/MS [1] with slight modifications. PA concentrations below limit of detection (LOD) were set to zero, while values between LOD and limit of quantification (LOQ) were set equal to „0.5*LOQ“. PA transfer rates were obtained by dividing total PA excretion into milk by PA exposure. All data were statistically analysed using the MIXED procedure for repeated measurements of the SAS software (version 9.4; SAS Institute Inc., Cary, NC, USA). Least square means were declared as significant if p-values were ≤0.05 after Tukey adjusted *t*-test (PDIFF).

Results: DM intake, milk yield, BW and general health were not significantly influenced by the applied PA exposure scenario [3]. The composition of the different PAs of milk differed from PA pattern of the extract. The main components in the PA-extract were Jaconine (34%), Seneciphylline-NO (7.5%), Jaconine-NO (7.5%), Seneciphylline (7.4%), Senecionine-NO (6.9%) and Jacobine (6.4%). In milk, however, Jacoline, Jaconine and Jacobine were the most abundant PAs with concentrations up to 16.1 µg/l (77%), 1.91 µg/l (9.1%) and 1.59 µg/l (7.6%) in group PA3, respectively. The transfer rate of total PA was 0.05%, 0.06% and 0.06% for groups PA1, PA2 and PA3, respectively, and differed not significantly (p=0.831).

Conclusions: The milk PA contamination was dominated by the PAs in their free base form while the composition of the PA-extract was more divers. There was no dose-dependent effect of the transfer of total PAs into milk as suggested by similar transfer rates. As PAs are cancerogenic substances the exposure of consumers has to be minimized by avoiding PA containing plants in feed and, consequently, the transfer of PA into milk.

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90.

The effects of microplastic on ruminal dry matter degradation *ex vivo*

Der Effekt von Mikroplastik auf die ruminale Abbaubarkeit von Trockenmasse ex vivo

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High amounts of microplastics accumulate in European soil yearly [1]. Recently, we tested the effects of different kinds of microplastic on targeted [2] and untargeted ruminal metabolomics *ex vivo* that suggest plastic-dependent depression of metabolic activity and plastic-specific clustering of ruminal metabolites. In order to extend our dataset, this study examined the effects of different chemical species of microplastics, applied in different particle sizes and dosages on the ruminal degradation of feed dry matter *ex vivo*.

Methods: The experiment was performed using the Hohenheim Gas Test in six consecutive experimental runs as described before (3). The microplastic species polylactide (PLA), polyhydroxy butyric acid (PHB), high-density polyethylene (HDPE), polyvinyl chloride (PVC) and polypropylene (PP) were applied in different average particle size ranges (<125 µm and 125-500 µm, respectively), at dosages of 0, 0.5, 5, 35 and 70 mg/incubation cylinder. The added microplastic thus represents 0%; 0.2%; 2%; 14% and 28% of the weighed dry feed mass in each cylinder. The different microplastic variants (microplastic species*particle size range*dose) were incubated (24h, 39°C, 1rpm) in addition of either 250 mg of dried barley or hay as fermentable feed materials each for three consecutive incubation runs, respectively. After incubation, cylinders were placed on ice to stop the fermentation immediately. Cylinder contents were sampled without losses and freeze dried to determine total dry matter. Statistical analysis comprised multifactorial ANOVA (plastic source, particle size, dose, feed) including interactions.

Results: In general, the dry mass degradation was significantly affected by microplastic source ($p = 0.04$) and dose ($p < 0.0001$) as well as feed ($p < 0.0001$) independently of each other. Regarding the source effect, PHB showed a decreasing effect on dry mass degradation, PVC a decreasing trend, whereas PP showed higher dry mass degradation compared to PLA, HDPE and the negative control without added microplastics. Furthermore, within higher added microplastic dose, the dry matter degradability decreased. The different average particle size distributions in the applied size ranges had no significant effect on the measured parameters. Overall, the findings of differential dry matter degradation with respect to the source of microplastic agree with Eichinger et al. [2], describing significant effects on short chain volatile fatty acid concentrations as well as on the cumulative gas production according to various microplastic species. Finally, barley-treated incubations showed the highest dry matter loss after 24h compared to hay-treated incubations, in each case irrespective of plastic source, dose, or partice size.

Conclusions: In conclusion, our data shows a microplastic source and dose dependent effect on the dry matter degradation *ex vivo*. Hence, microplastics negatively affect ruminal fermentation in a yet unclear mode-of-action. Future studies are necessary to identify the exact metabolic pathways in the rumen affected by microplastics. In particular, the animal level must be taken into account in order to be able to examine possible microplastic accumulation in the body, their consequences for animal health as well as the fate of microplastic within the ruminant's digestive tract.

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91.

Comparative ruminal metabolism of pyrrolizidine alkaloids in sheep and cattle

Vergleich des Pansenstoffwechsels von Pyrrolizidinalkaloiden bei Schafen und Rindern

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The growth of tansy ragwort (*Jacobaea vulgaris G. syn. Senecio jacobaea L.*) on pastures is observed with concern because it contains hepatotoxic and potentially carcinogenic pyrrolizidine alkaloids (PA/PANO). Therefore, agricultural use of ragwort-contaminated cropland could pose a risk to ruminants. Differences in the susceptibility to PA toxicity are reported between ruminant species, with cattle being more susceptible than sheep [1]. Our previous study invastigated the ruminal metabolism *in vitro*. The degradation rates for individual Senecio PA/PANOs were studied and respective ruminal metabolites identified [2]. In the work presented here, ruminal liquids of sheep and cattle were investigated which were fed with different doses of Senecio extracts.

Methods: Rumen liquid samples from two feeding experiment were analyzed by LC-MS/MS for nine typical Senecio pyrrolizidine alkaloids in their free base (PA) and N-oxide (PANO) form, as well as for 36 ruminal metabolites identified in previous work [3]. Both feeding experiments were conducted within the PA-SAFE-FEED project and contained four subgroups with four female lactating animals each: three different dose groups and one control group (molasses). The three dose groups received the following oral doses of an extract of Jacobaea vulgaris daily for a period of 28 days: sheep: 1.5, 3 or 6 mg/kg bw/d; cattle: 0.47, 0.95 or 1.91 mg/kg bw/d. Due to the high volume, the PA/PANO-bolus for the sheep was split into two daily administrations. Ruminal fluid of cattle was taken 1.5 h after administration at days 0, 7, 14 and 28 of the *in vivo* trial, and that from sheep was collected 1 h after administration at days -7, 7, 14 and 21. Significant differences were calculated via ANOVA with SPSS 26.0.0.1 (IBM, Armonk, New York).

Results: In previous *in vitro* experiments we demonstrated that PANOs are rapidly converted to their corresponding PAs, followed by biotransformation to metabolites in which the double bonds have been reduced, resulting in completely saturated structures. The degradation of the PAs to these saturated metabolites showed differences in kinetics, with jacoline degraded barely and jaconine and senkirkine degraded slowly compared to the other Senecio PAs [2]. As expected from these *in vitro* results the ruminal liquids from cattle and sheep in the feeding study contained no PANOs and only low amounts of PAs. Although the sheep received higher PA/PANOs doses compared to cattle and although ovine rumen samples were taken earlier, a lower PA concentration was detectable in the rumen fluid of the sheep. In both species, mainly jacoline was detected as 1,2 unsaturated PA although this was a minor PA in the administered PA/PANOs extract. With regard to the analytical scope the vast majority of the detected compounds were saturated metabolites in both species and even the concentration of these saturated metabolites was much lower in sheep compared to cattle.

Conclusions: Rumen metabolism of Senecio PA/PANOs for cattle and sheep revealed biotransformation to metabolites hydrogenated at the necine base moiety. As the double bond is generally considered as the precondition for PAs to exert their liver toxicity, rumen metabolism can be considered a detoxification step. Comparison of dose groups of both species showed that the concentration of PAs and their saturated ruminal metabolites is significantly lower in rumen of sheep than in cattle, without taking into account different ruminal retention times or volumes and other potential influences. The higher biotransformation rates in sheep compared with cattle might result in a more effective prevention of PAs adsorption in the digestive tract and thus in a reduced susceptibility [1, 2]. Also, ruminal metabolism could influence the carry-over processes such that fewer PAs and more metabolites are transferred to animal-derived foods.

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92.

Oral intake of sycamore seedlings by grazing dairy cows on pasture and detection of Hypoglycin A metabolites in milk

Orale Aufnahme von Keimlingen des Berg-Ahorns durch Milchkühe auf der Weide und Nachweis von Hypoglycin A Metaboliten in der Milch

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Hypoglycin A (HGA) and methylencyclopropylglycine (MCPrG) are secondary plant metabolites occurring in seeds and seedlings of sycamore maple trees (*Acer pseudoplatanus*). In humans and horses severe intoxications have been reported due to interference with the body´s energy metabolism. In contrast, ewes and their lambs seem less sensitive to intoxication with maple toxins [1]. The metabolic active forms of HGA and MCPrG, methylencyclopropylacetyl – CoA (MCPA-CoA) and methylencyclopropylformyl – CoA (MCPF-CoA), respectively, are conjugated with carnitine or glycine followed by elimination via urine [2]. Recently, traces of HGA were detected in a bulk milk sample from a dairy farm, indicating an ingestion of seedlings or seeds of sycamore maple trees by dairy cows and a transfer of maple toxins into the milk [3]. To date, though, it is not known if dairy cows voluntarily ingest sycamore seedlings, thus it was the aim of this study to test the hypothesis that dairy cows ingest seedlings from sycamore maple trees while grazing on a pasture and to investigate a possible transfer of maple toxins into milk.

Methods: Over 4 days at the onset of the grazing season in 2022, five individuals (n=5) out of a herd of 87 lactating cows, with an average milk yield of 21.3 ± 6 kg were given access to a pasture with two sycamore maple trees (1750 m^2). The pasture was exclusively used for grazing from 11 am to 3 pm on day 1 to day 4. On day 0, seven squares (50 × 50 cm) were set up distributed among the pasture. Four squares contained sycamore seedlings, while the remaining three squares were devoid of sycamore seedlings and only served as grazing control. Squares were photographed daily at 10.30 am and seedlings were counted before cows were allowed to graze. Animals were observed by two independent observers in a distance of 2 m and oral uptake of seedlings was randomly documented by video recording. The health status of study cows was checked daily by routine veterinary observations. Cows were milked twice daily and milk samples were taken from each study cow and from the bulk tank. On day 3, urine samples were collected from all 5 cows. Further, 500 g of Acer pseudoplatanus seedlings(both in two-leaf as well as four-leaf stage) as well as 500 g of the main vegetation were sampled representatively among the pasture and analyzed for Weende food nutrients. Moreover, seedlings, milk and urine samples were analyzed for contents of HGA, MCPrG and their metabolites using an inhouse-validated LC/MS-MS method at the BfR´s national reference laboratory for mycotoxins and plant toxins in feed and food.

Results: Cows voluntarily ingested sycamore seedlings containing on average 2.6 g/kg DM HGA, 0.2 g/kg DM MCPrG. Cows did not show any signs of discomfort or disease during the whole experiment neither their milk yields altered at any time. MCPA-Glycine and in some cases MCPA-Carnitine were detected in milk samples of individual cows. MCPA-Glycine was also detected in bulk tank milk samples from the whole herd (n=87). On day 3 and 4, MCPF-Glycine was detectable in individual milk samples. Traces of HGA in milk were below limit of quantification (LOQ). Urine samples showed glycine metabolites of HGA and MCPrG in all 5 cows.

Conclusions: Cows graze seedlings without developing signs of discomfort and rapidly metabolized and excreted HGA and MCPrG. Wether cattle are perhaps less susceptible to HGA intoxication is yet unknown. To quantitatively evaluate the transfer of maple toxin metabolites into milk and investigate possible biotransformation further data are required.

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93.

Determination of Mineral Oil Hydrocarbons in calves and piglets milk replacer by LC-GC-FID and GC×GC-ToF-MS

Bestimmung von Mineralölkohlenwasserstoffen in Kälber- und Ferkelmilchaustauschern mittels online-LC-GC-FID und GC×GC-ToF-MS

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Mineral oil hydrocarbons (MOH) are a complex mixture of substances divided into two main fractions. Mineral oil saturated hydrocarbons (MOSH) are composed of linear and cyclic branched, alkyl-substituted alkanes, whereas mineral oil aromatic hydrocarbons (MOAH) include mainly alkylated aromatic hydrocarbons with varying numbers of fused rings. Some MOSH are considered to accumulate in the human body following exposure, whereas MOAH are suspected to be mutagenic and carcinogenic substances. Calves and piglets are frequently fed with milk replacers. Since pathways of MOSH or MOAH along the food chain are hardly described, accumulation in food producing animals should be prevented. Following recent findings indicating the presence of MOAH in infant formula and follow-on formula, the aim of this study was to investigate whether MOH contamination would also occur in milk replacers frequently used in animal production.

Methods: Recently, an analytical method based on online-coupled liquid chromatography gas chromatography flame ionization detection (LC-GC-FID) was developed for the detection of MOAH in infant formula. This method was applied to selected samples of piglet and calves milk replacers. The two most common piglet milk replacers (stored in two different ways) and four common commercial calf milk replacers with different fat and protein sources were chosen for the analysis. Based on the number of samples and influencing factors, results were analyzed descriptively.

Results: Both, MOSH and MOAH were detected in all analyzed samples with varying quantities. Subsequent analysis of the individual MOSH and MOAH fractions by comprehensive two-dimensional gas chromatography coupled to mass spectrometry (GC×GC-ToF-MS) revealed that MOSH were accompanied by polyolefinic saturated hydrocarbons (POSH), whereas MOAH were mainly constituted by one- and two-ring aromatic hydrocarbons. Further analysis revealed that POSH were presumably transferred from the plastic storage containers of the milk replacer samples. The source of the contamination with MOH is currently unknown, and will be further investigated.

Conclusions: Our results indicate that MOH contamination of milk replacers seems to occur and is highly dependent on the used storage containers. Furthermore, online-LC-GC-FID can provide an adequate analytical tool for quantification of MOH in this particularly complex food matrix.

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94.

Water-associated physical properties – a standardized approach for the evaluation of feeds

Wasserassoziierte physikalische Eigenschaften – einheitliches Vorgehen zur Bewertung von Futtermitteln

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In addition to chemical characterisation using a standard procedure, current scientific studies are increasingly focusing on the assessment of physical properties. In particular, measurement and integration of the water-associated physical properties of feeds in diet calculation, such as water holding (WHC) and binding capacity (WBC) as well as swelling properties (SP) have to be given more consideration in the future. One reason is due to varying physiological effects that can be expected along the gastrointestinal tract due to WHC, WBC and SP. Furthermore, close correlations between the content of several nutrients and fibre fractions (soluble/insoluble) with water-associated properties have been observed [1]. Currently, a major challenge lies in the application of different procedures for the methods with regard to the determination of mentioned properties and terminology (especially for WBC and WHC). These circumstances reduce comparability of results between laboratories and thus allow a classification of feeds only in a very limited way. In order to gain comparable results, the aim of the present study was to compare analytical methods for WHC, WBC and SP, to define a standardized approach and to verify these methods.

Methods: A collaborative study was designed including 16 laboratories of Germany and Austria (VDLUFA, 487M). Each laboratory got a standardized sample of soybean hulls (SBH) as well as a sample of conventional fattening pig diet which were analysed for WHC, WBC, SP and dry matter (DM) according to the adjusted instructions [modified after 1, 2, 3]:

(1) WHC: 5.0 g sample (E, grinded to pass a 1.0 mm sieve), was soaked in a beaker (50 mL high-rise) with 30 mL distilled water for 24 h, stirring every 30 min within first 2 h. A funnel with filtration paper (PTF, dried at 103 °C ± 2 °C, pore size 12 µm) was soaked with water and subsequently covered with a watch glass for 2 h (PFF). Afterwards soaked sample was filtrated, washed tree times with additional water to avoid residues and covered with a watch glass for 2 h (PFP). Finally, filtration paper with sample was dried (PTP, 103 °C ± 2 °C) and WHC was calculated according to equation: WHK = ([PFP - PFF] - [PTP - PTF]) ·100/[E · DM]

(2) WBC: 3.0 g sample (E, grinded to pass a 1.0 mm sieve) was soaked with 30 mL distilled water (24 h) in a centrifuge glass tube (PZ, >50 mL) stirring every 30 min within first 2 h and centrifuged (3000 x g for 20 minutes at room temp.). Afterwards, the unbound water was decanted, glass tube turned upside down for 10 min and finally dried (PT, 103 °C ± 2 °C). WBC was calculated according to equation: WBK = [PZ - PT] ·100/[E · DM]

(3) SP: 5.0 g sample (E, grinded to pass a 1.0 mm sieve) given in metric cylinder (100 mL; 1.0 mL subdivisions) (V1) was soaked with 40 mL distilled water (24 h, V2), stirring every 30 min within first 2 h. Swelling was expressed as proportion of the final volume (V2 after 24 h) and the initial volume (V1): QV = V2/V1

Results: Analytical results of all 16 participating laboratories were submitted and evaluated. For SBH mean value for WHC was 6.12 g/g DM and tolerance range (TR) was from 5.45 to 6.83 g/g DM with RSD of 5.60%, while mean value of WBC was 5.56 g/g DM with a TR from 5.06 to 6.08 g/g DM and RSD of 4.61%. Diet of fattening pigs resulted in a mean value of 2.09 g/g DM for WHC, with TR from 1.77 to 2.44 g/g DM and RSD of 7.93%. Mean value of pig´s diet for WBC was 1.55 g/g DM and TR was between 1.27 to 1.86 g/g DM with RSD of 9.36%. Regarding the SP, SBH showed mean values of 3.47 with RSD of 7.81% and pig´s diets of 1.96 with RSD of 7.75%. TR of SBH ranged from 2.95 to 4.03 and of pig´s diet from 1.67 to 2.28.

Conclusions: The applicability of methods was confirmed in the collaborative study. However, minimal adjustments of method descriptions are necessary to guaranty the comparability between results of different laboratories. Finally, an implementation as VDLUFA methods in the VDLUFA method manual is planned in 2023.

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95.

Effect of grain:water ratio on some fermentation characteristics of rye and sorghum

Einfluss des Mischungsverhältnisses von Getreide und Wasser auf einige Fermentationseigenschaften von Roggen und Sorghum

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A promising method that can be applied to improve the nutritive quality of some alternative protein sources is the fermentation process, resulting in improvement of digestibility [1]. For optimal fermentation, carbohydrate rich feedstuffs such as cereal grains are also necessary as the glucose generated is a preferred substrate for fermentative microbes [2]. Therefore, fermentation of alternative protein sources requires co-fermentation with grains, and hence can lead to overcoming negative properties through the action of fermentation itself. This study was conducted to determine some fermentation characteristics of rye or sorghum mixed with different ratios of water (25%, 37.5%, 50%, 62.5%, and 75% in dry matter (DM)), incubated up to 48 h *in vitro*.

Methods: Hybrid rye (880 g/kg DM) and sorghum (860 g/kg DM) grains were included in this study. The ground grains were fermented *in vitro* by using a 300 mL beaker. Briefly, the sample-to-water ratio was adjusted to obtain the planned DM contents (25%, 37.5%, 50%, 62.5%, 75%) in the mixture before fermentation. To avoid malfermentation, a freeze-dried, granulated starter culture (Schaumalac Feed Protect XP G, H. Wilhelm Schaumann GmbH, Pinneberg, Germany), was added at the beginning of each fermentation process at a dose of 2×10^7 CFU/g ingredient. Samples were taken during and after fermentation at 0, 6, 12, 24 and 48 h for further analysis. The pH values in the collected samples were determined directly. Samples were obtained from each grain with different DM contents at three time points for lactic acid bacteria counts. Moreover, further samples were taken for determination of L-lactic acid, fatty acid and viscosity values. All parameters were analyzed for the individual samples ($n = 4$). Moreover, the mean values as well as the standard deviation of the mean were calculated. Differences with a significant level of $p < 0.05$ were considered significant.

Results: After 24 h of fermentation, significant differences in the particle size distribution <0.20 mm among the different DM contents of rye were noted. Whereas fermented rye with 37.5% DM had the greatest particle size distribution <0.20 mm (73.9%), fermented rye with 62.5% DM content had the lowest (46.4%). While, in sorghum, after 24 h of fermentation, only significant differences were apparent in the particle size distribution <0.20 mm between fermented sorghum of DM contents of 62.5% and 75% (24.2 and 17.5%, respectively). The pH of the fermented rye at a DM content of 25% after 24 h had the lowest values (3.57) compared to that at a DM content of 75% (6.42). In fermented sorghum, pH values were lower than 4 already after incubation at 25% DM for 12 h (3.93) in comparison to that at DM content 75% (6.51). The L-lactic acid concentration in the fermented rye with 25% DM content after 24 h was significantly the highest (18.7 g/kg DM), as was that of sorghum of 25% DM content after 24 h (22.2 g/kg DM). Moreover, the acetic acid level in the fermented rye with 25% DM content after 24 h was significantly the highest (3.02 g/kg DM) compared to the other DM contents of fermented rye. Also, in fermented sorghum (25% DM), the acetic acid content was significantly the greatest (1.49 g/kg DM) in comparison to the other DM contents of fermented sorghum.

Conclusions: Overall, fermented rye and sorghum containing 25 or 37.5% DM for 24 h and 12 h for rye and sorghum, respectively are sufficient for fermentation to be optimized based on the values of pH and lactic acid content. However, fermented rye and sorghum containgning 25% DM had significant high content of acetic acid which may negatively affect the palatability in animals. The present study confirmed that LAB can ferment rye and sorghum, particularly with 25% and 37.5% DM, successfully and demonstrated that both grains undergo rapid fermentation.

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96.

Social hierarchy influences the drinking behavior of dairy cows at a trough near the milking parlor after milking

Die Rangordnung beeinflusst das Trinkverhalten von Milchkühen an einem Melkstand-nahen Trog nach dem Melken

*Wahlen R., Burkhardt F. K., Steinhoff-Wagner J. – Freising

The absence of prolonged thirst is a major welfare indicator of the Welfare Quality® protocol in dairy cows (Welfare Quality®, 2009) and one of the most powerful indicators used to classify animal welfare at the farm level (Heath et al., 2014). Social rank seemed to be a limiting factor for access to water in dairy herds (Burkhardt et al., 2022). Therefore, the aim of this study was to evaluate the influence of social hierarchy on dairy cows drinking behavior after milking at a trough near the milking parlor.

Methods: This study was conducted at an experimental barn (Veitshof, Freising) with a herd of 44 lactating Brown Swiss cows that were held in a free-range barn. Cows were milked and fed twice a day in the morning and evening. The drinking behavior at a milking parlor facing valve trough (0.36 m length, 0.32 m wide, 0.10 m depth; variable volume) made of stainless steel was recorded twice daily by video during the milking until the last cow left the milking area. The study was conducted over a period of 11 d in July 2022 and the consumed volume of water was measured using a calibrated water meter with roller counter. Social rank was defined as the order of cows enforcing their access to the milking parlor. Each drinking episode was characterized using Behavioral Observation Research Interactive Software (BORIS) for counting the total numbers of 'drinking episodes', 'sips per drinking episode, 'tasting behaviors', 'swallowing difficulties', 'agonistic behaviors', 'drinking breaks' and 'interruptions of the drinking episode due to agonistic behavior' as well as the total duration of a 'drinking episode', a 'tasting episode', a 'drinking breaks' and a 'water intake' period. The recorded behavioral parameters were analyzed using a linear model with post hoc Tukey correction and social rank (quartiles of the rank) as the fixed factor and Spearman rank correlations in SAS (SAS 9.4).

Results: The most dominant quartile of cows drank most frequently immediately after milking (14.0 ± 1.7 drinking episodes) whereas the dominants (8.7 ± 1.7), subordinates (8.7 ± 1.3), and the most subordinates (8.8 ± 1.8) had less drinking episodes ($P < 0.05$) and were more often interrupted by other cows while drinking ($P < 0.01$). The lower the social rank, the higher the occurrence of not drinking immediately after milking ($r = -0.3$, $P < 0.05$). The rank position was correlated positively with observations that other cows were pushed aside due to agonistic behavior ($r = 0.4$, $P < 0.05$).

Conclusions: This study indicates that dairy cows' drinking behavior are influenced by the social rank of the individual within the herd. Subordinate cows seem to avoid drinking at the nearest trough to the milking parlor as they visited it less often. Further research on the effects on dairy cows' drinking behavior might be useful to optimize water provision on dairy farms and thereby improve animal welfare.

Acknowledgement: Thanks to the staff of the TUM Animal Research Center working at the Veitshof for their support.

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97.

Effects of the drinking water applications LOVIT Calcifit Select and LOVIT Phos Plus Liquid on performance of laying hens

Auswirkungen der Trinkwasserapplikationen LOVIT Calcifit Select und LOVIT Phos Plus Liquid auf die Leistung von Legehennen

*Kubitza D., Hagen-Euteneuer N., Zentek J. – Cuxhaven / Berlin

As calcium is the main constituent of the eggshell, it is a crucial component in the hens' diet. At the same time, a balanced supplementation with phosphorous and vitamin D needs to be provided in order to allow proper metabolization and release of calcium and consequently mineralization of bones and eggshell. Especially in times of higher demand, supplementation via feed alone may not be sufficient. Hence, application of these nutrients via drinking water can be a fast solution to cover the animals' requirements and maintain high levels of performance.

Methods: The study comprised 90 hens from day 255 to day 344 of age allocated to two treatment groups in 30 pens (3 hens per pen; 45 hens per treatment). While the first treatment group (T1) did not receive any supplemental additives in the drinking water, hens from the second treatment group (T2) received LOVIT Calcifit Select (Kaesler Nutrition; effervescent granules, containing vitamin D_3, calcium and zinc) once a week at a dosage of 1 g/l and LOVIT Phos Plus Liquid (Kaesler Nutrition; liquid formulation, containing phosphorus, calcium, magnesium, sodium, manganese, zinc, and copper) at a dosage of 1 ml/l three times a week in the drinking water. Efficacy was demonstrated on laying performance (per 30-day intervals), apparent total-tract digestibility (4-day collection period from day 87 to 90), and tibia mineralization (analyzed on day 90). All data were analysed by analysis of variance according to completely randomized design using the software package SPSS (IBM SPSS Version 25). Multiple comparisons between treatment groups were made by Tukey's and significant differences were declared at $P \leq 0.05$. Outliers, if any, were not removed prior to statistical analysis.

Results: Hens receiving LOVIT additives via drinking water showed a significantly increased overall laying rate (+1.26%; $P < 0.04$.) and overall egg-mass output (+3.37%; $P < 0.027$). At the same time, the overall feed-to-egg mass ratio tended to be significantly reduced (-4.36%; $P < 0.075$). There was no difference among the two treatment groups in terms of percentage of broken or dirty eggs, egg quality, and egg yolk colour. Strikingly, egg shell breaking strength was improved from day 60 onwards and reached levels of statistical significance on day 90 of the trial (+4.88%; $P < 0.011$). In addition, the apparent total-tract digestibility of crude ash, calcium, and phosphorous showed on average improved values compared to the control group (+6.47%). In line with this, defatted tibia bones contained higher amounts of crude ash, phosphorous, and calcium with the latter tending to be significantly different from the control group (+7.10%; $P < 0.074$).

Conclusions: The results demonstrate that the application of LOVIT Calcifit Select and LOVIT Phos Plus Liquid according to the treatment scheme was able to significantly improve laying rate and egg mass. Apparent total-tract digestibility and tibia mineralization were improved during the course of the trial, which was also reflected by an increased egg shell breaking strength in the trial group. However, changes in egg shell breaking strength were not detectable in the first 30 days while laying performance already improved during this period.

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